SCIENCE LIBRARY

179

JUL 5 1929

VOLUME 4 NUMBER 2

PLANT PHYSIOLOGY

APRIL, 1929

CONTENTS

A Study of the Relations Between Chloroplast Pigments and Dry Weight of Tops in Dent Corn. H. B. SPRAGUE and J. W. SHIVE. New Jersey Agricultural Experiment Station 165

The Toxic Substance Produced by the Eye Spot Fungus of Sugar Cane, Helminthosporium sacchari Butler. ATHERTON LEE. Philippine Sugar Association, Manila, P. I. 193

The Absorption of Phosphate from Soil and Solution Cultures. L. J. H. TEAKLE, Department of Agriculture, Perth, Western Australia 213

The Extraction of Nitrogenous Materials from Pear Tissues. F. B. LINCOLN and A. S. MULAY, University of California 233

Hemicellulose as a Storage Carbohydrate in Woody Plants, with Special Reference to the Apple. A. E. MURNEEK, University of Missouri 251

Identification of Certain Species of Citrus by Colorimetric Tests. F. F. HALMA and A. R. C. HAAS, University of California 265

The Effect of Potassium, Nitrogen and Phosphorus Fertilizing upon the Chloroplast Pigments, upon the Mineral Content of the Leaves, and upon Production in Crop Plants. F. M. SCHERTZ, United States Department of Agriculture 269

The Effect of Temperature on Flowers. F. M. ANDREWS, Indiana University 281

Brief Papers. Wilhelm Pfeffer. F. M. ANDREWS, Indiana University. *A Simple Method for Nitrate Nitrogen Determination in Wheat Plants.* H. F. HOLTZ and CARL LARSON, State College of Washington 285

Notes 293

PUBLISHED QUARTERLY
BY
THE AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS
AT LIME AND GREEN STREETS, LANCASTER, PA.
THE SCIENCE PRESS PRINTING CO.

Entered as second-class matter March 25, 1926, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

PLANT PHYSIOLOGY

A QUARTERLY JOURNAL
DEVOTED TO ALL PHASES OF PLANT PHYSIOLOGY

Established 1926

EDITORIAL BOARD

CHARLES A. SHULL, *Editor-in-Chief*,
The University of Chicago

CHARLES B. LIPMAN,
The University of California,
Berkeley, Calif.

BURTON E. LIVINGSTON,
Johns Hopkins University,
Baltimore, Md.

CARLETON R. BALL,
U. S. Department of Agriculture,
Washington, D. C.

FRANCIS E. LLOYD,
McGill University,
Montreal, Canada.

Assisted by the officers of the American Society of Plant Physiologists.

The pages of this journal are open to papers of merit dealing with any phase of plant physiology. Membership in the Society is not required of contributors.

Manuscripts must be typewritten, and must have the approval of the Editorial Board. In the preparation of papers, illustrations, diagrams, and tabulated data should be used sparingly, and only when necessary to proper presentation.

Proofs should be returned to the Editor-in-Chief promptly, with necessary corrections, and reprints should be ordered when the proof is returned. Until further notice reprints will be furnished with or without covers at cost of production. Twenty-five copies without covers are sent gratis provided the author has placed an order for reprints.

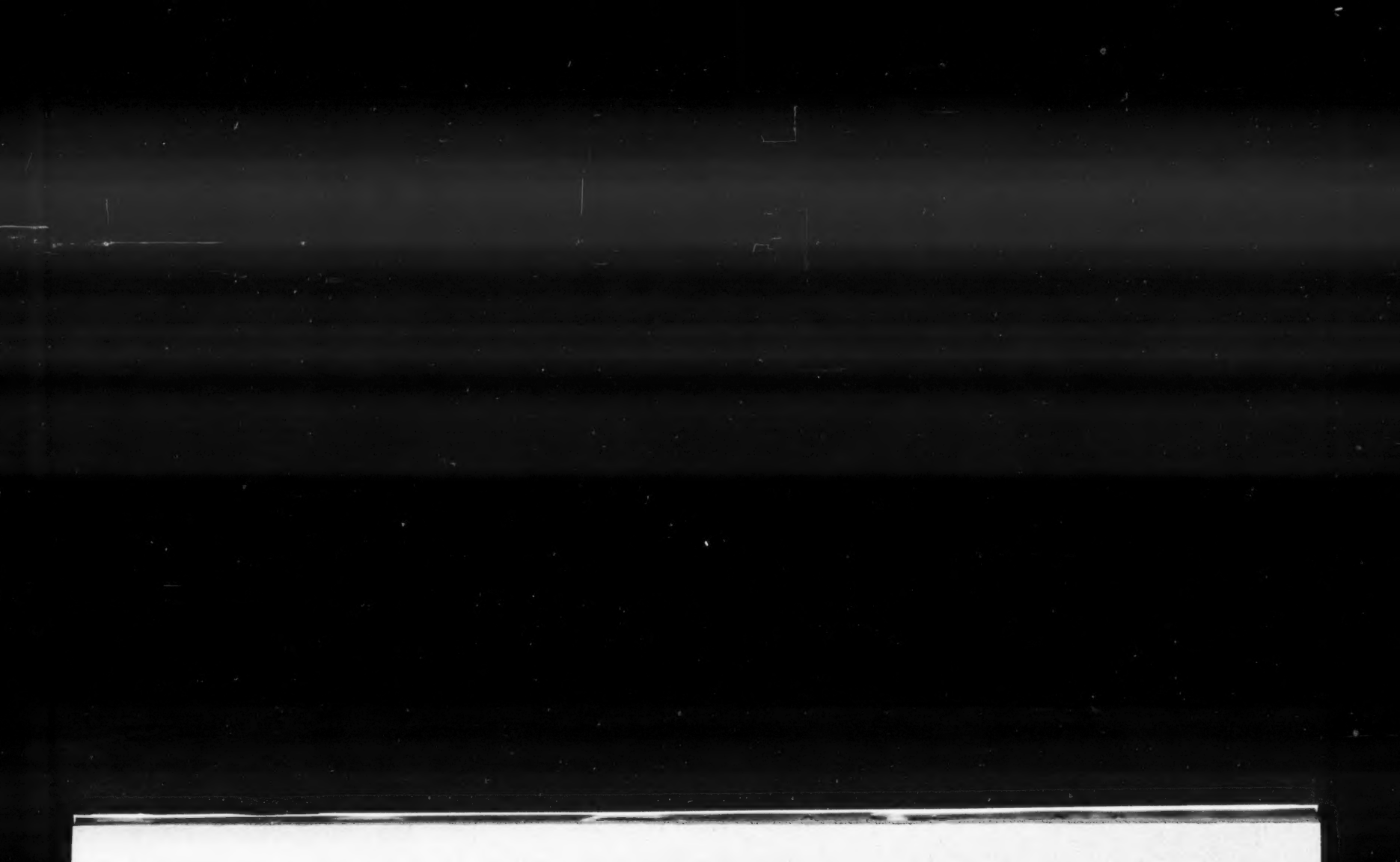
Correspondence with reference to editorial matters should be addressed to the Editor-in-Chief.

Subscription price, $8.00 a year. Single copies, $2.50 post free. Members of the American Society of Plant Physiologists receive the journal in connection with dues paid to the Society.

Missing numbers will be supplied if lost in the mail, provided that written notice is received within one month of date of issue. Otherwise they will be furnished at the single copy rate, as long as they are available.

Remittance should be made payable to PLANT PHYSIOLOGY.

Business correspondence regarding subscriptions, changes of address, back numbers, and discontinuances may be addressed to Lime and Green Sts., Lancaster, Pa., or to Henry R. Kraybill, Department of Agricultural Chemistry, Purdue University, Lafayette, Indiana.



Vol. 4 No. 2

PLANT PHYSIOLOGY

APRIL, 1929

A STUDY OF THE RELATIONS BETWEEN CHLOROPLAST PIGMENTS AND DRY WEIGHTS OF TOPS IN DENT CORN[1]

H. B. Sprague and J. W. Shive

The dearth of information regarding the exact relation between the concentration of the chloroplast pigments (chlorophyll α and β, carotin, and xanthophyll) in higher plants, and growth rates, and between the total quantity of these pigments and the total amount of growth made (**10**) (**12**), was the stimulus which started the work here reported. Three series of experiments were conducted, to determine as carefully as possible the amounts of chloroplast pigments present in certain leaves at periodic intervals, as well as leaf areas and dry weights of the plant tops. Dent corn (*Zea mays* L.) was selected for the tests, because of its great economic importance and the availability of practically homozygous seed.

Experimental methods

Three series of plants were grown, series 1 and 2 in the greenhouse in the springs of 1925 and 1926, and series 3 in the field during the summer of 1925. Nebraska 10 and Nebraska 12, two lines of corn that had been self fertilized for 17 generations, were used in all experiments. Schmidt's White Cap, a local open-pollinated strain was included in series 1. The F_1 cross between the selfed lines Nebraska 12 and Nebraska 10, was used in series 2, and the F_1 cross of Nebraska 12 and Nebraska 659 in series 3. The use of selfed lines and of first generation crosses avoided much of the random variation among individual plants that would have been present had ordinary varieties been selected. At the same time, it provided for constant genetic differences between the plants of the different strains. All of the strains chosen appeared normal for color.

The seeds planted were carefully selected for uniformity. Those of the selfed lines and the crosses planted in series 1 and 2 weighed between 0.185 and 0.195 grams, and those of Schmidt's White Cap averaged 0.295 grams.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Physiology.

The seeds planted in series 3 were selected for uniformity of size but were not weighed.

Series 1 and 2

The plants in series 1 and 2 were grown in percolators, as described by Allison (**1**), each filled with 3,328 grams of pure washed quartz sand. The seeds were germinated in sphagnum moss and six seedlings were transplanted to each percolator when the plumules were 2 cm. long in series 1, and 3 to 4 cm. long in series 2. Culture solution was supplied by the constant drip method (**9**). Beginning 30 days after transplanting in series 1, and 12 days after transplanting in series 2, distilled water was added once daily to the surface of the sand in each percolator just before renewing the solution in the constant drip reservoirs. This largely prevented concentration of the nutrient solution in the sand through water loss by absorption and evaporation. Enough water was added to cause free dripping from the bottoms of the percolators.

The plants in series 1 were supplied with Shive's nutrient solution R_5C_2 (1.75 atm.) at the rate of 1 liter per percolator per day. For series 2, Tottingham's (**13**) solution $T_1R_1C_5$ as modified by Jones and Shive (**3**) was used at the same rate. Iron was added to the nutrient solution daily in the form of soluble ferric phosphate. The amount used was varied as needed to maintain a normal green color. For series 1, a total of 446 mg. of iron was added to each culture of Nebraska 12 and Schmidt's White Cap, and 334 mg. to Nebraska 10 during the growth period of 52 days. For series 2, a total of 101.7 mg. of iron was added to each culture of Nebraska 12 and the F_1 (Nebraska 12 x Nebraska 659) while 76.3 mg. were added to Nebraska 10 cultures, during the period of 45 days.

Series 3

Series 3 was planted in a well prepared seed bed on May 20. The soil was Sassafras silt loam, to which 400 lbs. of a commercial fertilizer having an analysis of 2 per cent. NH_3, 12 per cent. P_2O_5 and 2 per cent. K_2O was broadcast before planting. The crop was tilled in the ordinary manner except that care was taken to prevent injury to any seedlings and to prevent weed growth.

Sampling

The plants in each series were considered up when the first leaves began to unfold. The ages given in subsequent tables are on the basis of dates when plants came up. Plants were harvested periodically using 12 plants of Nebraska 12 and Nebraska 10, and 2–8 plants of Schmidt's White Cap at each harvest in series 1; and 6 plants per strain per harvest in series 2 and 3.

Leaf areas (leaf expanses) were obtained by means of blueprints and a planimeter for the first three harvests of series 1. All other leaf areas were determined by the formula (**4**):

$$\text{leaf area} = 0.75 \text{ (total length x width at widest place)}$$

Only the green portions of the blades were used in determining areas.

One leaf from each plant was selected for analysis of chloroplast pigments; namely, the uppermost one which had attained its maximum size and a normal color. The dry weight of each leaf used for pigment analysis was calculated by assuming that the ratio of area to weight was the same for this leaf as for the rest of the leaves on the plant. Although the uppermost fully developed leaves may not have been absolutely representative of all the leaves on that plant, they should have been comparable for all strains at each harvest since the same method of selection was used throughout.

Determination of chloroplast pigments

The fresh green leaves selected for pigment analyses were thoroughly triturated with sand to permit quantitative extraction. The method used in extraction and separation of the various pigments was that of Willstätter (**14**) as modified by Schertz (**7**). The chlorophylls, α and β, were obtained together as an aqueous solution of the chlorophyllins, which appeared green by transmitted light. In this work, it was found convenient to use petrol ether instead of the diethyl ether recommended by Schertz as a solvent for the carotinoids; the quantities of these pigments, therefore, were determined from petrol ether solutions.

The amount of each pigment was measured colorimetrically by comparing a solution of the pigment with an artificial color standard in a Duboscq colorimeter. The color standard for chlorophyll was prepared by adding 0.3 cc. of a 0.5 per cent. aqueous solution of malachite green and 11.2 cc. of a 0.5 per cent. aqueous solution of naphthol yellow to distilled water, and making up to 5,500 cc. volume. The carotin color standard was made by adding 3.4 cc. of a 0.5 per cent. aqueous solution of naphthol yellow and 0.5 cc. of a 0.5 per cent. aqueous solution of orange G. crystals to 1 liter of distilled water. The xanthophyll color standard was prepared by adding 3.8 cc. of the 0.5 per cent. aqueous solution of naphthol yellow to 1 liter of distilled water.

The 0.5 per cent. aqueous stock solutions of malachite green, naphthol yellow, and orange G. will keep for a long time in tightly stoppered bottles in the dark (table I). The color standards, however, should be freshly prepared from the stocks every one or two weeks to eliminate any danger of fading of colors. Stock solutions of the dyes should be discarded whenever a sediment appears.

TABLE I

THE STABILITY OF THE 0.5 PER CENT. STOCK SOLUTIONS OF DYES USED IN MAKING COLOR STANDARDS FOR CHLOROPLAST PIGMENTS[1]

DYES	FRESHLY MADE	6 MONTHS OLD	8 MONTHS OLD	10 MONTHS OLD	20 MONTHS OLD
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Naphthol yellow[2] (Martius yellow) Schultz No. 6	100.0	100.0	100.0	98.0	100.0
Orange G. Crystals[3]	100.0	64.5[4]	66.7[4]	100.0	100.0
Malachite Green[2] Schultz No. 495	100.0	100.0	100.0	100.0	100.0

[1] The stock solutions were diluted until readings could be made accurately with the Duboscq colorimeter.

[2] Purchased from the National Aniline and Chemical Co., Inc., New York.

[3] Purchased from Arthur Thomas Co., Philadelphia.

[4] Heavy sediment found in solutions.

Each color standard was evaluated in terms of milligrams of the pigment with which it was used. Dr. F. M. SCHERTZ, of the U. S. Department of Agriculture kindly provided a sufficient quantity of pure crystalline chlorophyll (α plus β) to evaluate the chlorophyll standard. Five hundred milligrams of chlorophyll were dissolved in acetone, transferred to petrol ether, saponified with methyl alcoholic potash in the usual way, separated from the ether solution, and made up to volume with water. This solution was then compared with the artificial chlorophyll color standard in the colorimeter. It was found that 10.708 milligrams of chlorophyll converted to chlorophyllins and made up to 1 liter matched the tint and depth of color of the artificial standard.

Evaluation of the carotin and xanthophyll standards was accomplished by extracting and purifying these pigments, and making readings with the colorimeter of the pigments in solution against their respective color standards. The concentration of pure pigment in each solution was determined from aliquot parts of the same solution by means of a König-Martens spectrophotometer at the Bureau of Standards at Washington, D. C. This was accomplished through the courtesy of Dr. D. S. GIBSON of that Bureau and of Dr. F. M. SCHERTZ of the U. S. Department of Agriculture. This quantitative method of measuring the carotinoid pigments has been worked out by SCHERTZ (**5, 6**). It was found that 1.890 milligrams of carotin per liter of solution were required to match the tint and depth of color of the carotin color standard, whereas 1.537 milligrams of xanthophyll per liter

of solution were required to match the tint and depth of color of the xanthophyll standard.

WILLSTÄTTER and STOLL (**14, 15**) prepared color standards for chlorophyll by making chlorophyllin solutions from known quantities of pure chlorophyll; and for the carotinoids by making up solutions of known concentrations of the pigment in question, or by making up solutions of potassium dichromate corresponding in color to solutions of a known concentration of the pigment to be measured. The color standards described above proved more satisfactory than WILLSTÄTTER's since they were easily prepared; also the colors were more stable, and were identical with those of solutions of the pigments to be measured. Since pure chlorophyll, carotin, and xanthophyll are difficult to prepare and may not be purchased in the open market, the artificial standards are quite useful when once prepared. The colorimetric method of measuring pigments is satisfactory only if consistent readings may be obtained. When stable color standards, which accurately match the colors of the solutions to be measured, are used, the colorimetric method is nearly as satisfactory as the spectrophotometric method and is much more readily available. Different lots of the dyes used in preparing the artificial color standards may vary in purity and color, which makes it necessary to evaluate each new lot as described above. However, 10 grams of each dye should supply the needs of one worker for several years.

After solutions of the pigments were separated from the green leaves for comparison with the color standards, the volume was increased until accurate readings could be made on the colorimeter. Five readings were made for each determination and the results averaged.

Experimental data

Data on the leaf areas, dry weight of tops, and the dry weight of tops per 100 square cm. of leaf area for all 3 series are given in tables II, III, and IV. In each series, plants of Nebraska 10 had smaller leaf areas and dry weights than those of Nebraska 12, and plants of Nebraska 12 were in turn smaller and lighter than those of Schmidt's White Cap or the F_1 crosses. It will be noted, however, that averaged dry weight per 100 square cm. of leaf area was lowest for plants of Nebraska 12, and that the leaves of Nebraska 10 were about equal to those of Schmidt's White Cap and the F_1 crosses in this respect.

Table IV also brings out the interesting fact that the length of time which leaves remain green is an important factor in determining yield at maturity. Nebraska 10 had a smaller leaf area and dry weight than Nebraska 12 during the first 49 days of growth, but at 96 days it had an equal leaf area. The lower leaves of Nebraska 10 remained green much

TABLE II

LEAF AREAS AND DRY WEIGHTS OF TOPS OF CORN[1] GROWN IN THE GREENHOUSE (1925) IN SAND CULTURES WITH SHIVE'S R_5C_2 NUTRIENT SOLUTION (SERIES 1)

DATE OF HARVEST	AGE OF PLANTS	LEAF AREA, AVERAGE PER PLANT			DRY WEIGHTS OF TOPS, AVERAGE PER PLANT			AVERAGE DRY WEIGHT PER 100 SQ. CM. OF LEAF		
		NEBRASKA NO. 10	NEBRASKA NO. 12	SCHMIDT'S WHITE CAP	NEBRASKA NO. 10	NEBRASKA NO. 12	SCHMIDT'S WHITE CAP	NEBRASKA NO. 10	NEBRASKA NO. 12	SCHMIDT'S WHITE CAP
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
	days	*sq. cm.*	*sq. cm.*	*sq. cm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
March 14	10	25.4	27.4	13.1						
March 21	17	63.0	90.2	108.1	0.185	0.236	0.266	0.158	0.134	0.128
March 28	24	144.7	221.3	201.9						
April 4	31	248.8	365.0	530.1						
April 11	38	400.9	558.5	968.8	1.962	2.747	6.031	0.258	0.233	0.305
April 18	45	684.6	840.3	1616.2	3.870	4.829	10.973	0.295	0.263	0.305
April 25	52	1017.5	1415.9	2401.8	6.417	8.371	19.625	0.317	0.268	0.377

[1] 12 plants were averaged in each case to get data on Nebraska 10 and Nebraska 12. The number of plants of Schmidt's White Cap used varied from 2 to 8; and they were 5 days younger throughout the series than indicated in Column 2.

TABLE III

LEAF AREA AND DRY WEIGHT OF TOPS OF CORN[1] GROWN IN THE GREENHOUSE, 1926, IN SAND CULTURES WITH A MODIFIED TOTTINGHAM SOLUTION $T_1R_1C_3$ (SERIES 2)

DATE OF HARVEST	AGE OF PLANTS	LEAF AREA PER PLANT			DRY WEIGHT OF TOPS PER PLANT			DRY WEIGHT PER 100 SQ. CM. OF LEAF		
		NEBRASKA NO. 10	NEBRASKA NO. 12	F_1[2]	NEBRASKA NO. 10	NEBRASKA NO. 12	F_1	NEBRASKA NO. 10	NEBRASKA NO. 12	F_1
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
	days	*sq. cm.*	*sq. cm.*	*sq. cm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
April 9	10	19.4	29.3	37.4	0.061	0.094	0.122	0.189	0.175	0.188
April 16	17	63.5	117.6	144.1	0.236	0.397	0.514	0.217	0.195	0.212
April 24	25	188.1	295.1	475.1	0.647	0.970	1.821	0.211	0.179	0.218
April 30	31	308.2	604.7	899.1	1.60	3.32	5.27	0.293	0.273	0.293
May 7	38	621.6	1203.0	1495.4	3.80	7.94	11.04	0.321	0.300	0.323
May 14	45	1233.7	1673.5	2179.4	8.81	14.44	22.83	0.357	0.348	0.392

[1] Six plants were averaged in each case to obtain data.

[2] The F_1 is a cross between Nebraska 12 (♀) and Nebraska 10 (♂).

TABLE IV

LEAF AREA AND DRY WEIGHTS OF TOPS OF CORN[1] GROWN IN THE FIELD DURING THE SUMMER OF 1925 (SERIES 3)

Date of harvest	Age of plants	Average leaf area per plant			Average dry weight of tops per plant			Average weight per 100 sq. cm. of leaf		
		Nebraska no. 10	Nebraska no. 12	F_1	Nebraska no. 10	Nebraska no. 12	F_1	Nebraska no. 10	Nebraska no. 12	F_1
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
	days	*sq. cm.*	*sq. cm.*	*sq. cm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
June 9	10	41.7	66.1	97.2	0.14	0.21	0.32	0.209	0.195	0.207
" 13	14	73.8	109.2	198.1	0.28	0.42	0.90	0.236	0.231	0.281
" 20	21	167.7	247.6	563.8	0.72	1.03	2.97	0.250	0.235	0.312
" 27	28	452.6	740.4	1559.8	2.58	4.44	11.65	0.337	0.305	0.377
July 4	35	840.2	1383.2	2773.7	5.70	7.90	18.43	0.363	0.314	0.365
" 11	42	2091.1	2934.9	5164.8	16.67	21.47	49.85	0.439	0.360	0.425
" 18	49	2943.7	4374.9	6650.9	34.10	51.70	94.65	0.507	0.462	0.507
					Total dry weight at maturity					
Sept. 3[2]	96	3859.6	3858.9	5698.3	204.9	166.9	328.7			
					Dry weight of shelled grain					
					90.1	79.2	182.1			
					Date of maturity					
					Sept. 8	Sept. 4	Sept. 7			

[1] The F_1 is a cross between Nebraska 12 (♀) and Nebraska 659 (♂).

[2] Leaf areas taken on September 3. Plants actually harvested September 15.

TABLE V

RATES[1] OF GROWTH AND ATMOSPHERIC CONDITIONS FOR CORN GROWN IN THE GREENHOUSE, 1925, IN SAND CULTURES (SERIES 1)

PERIOD	DAILY RATES OF INCREASE						DAILY ATMOMETER LOSS	MEAN DAILY TEMPERATURE FOR THE PERIOD	AVERAGE RELATIVE HUMIDITY AT NOON
	LEAF AREA			DRY WEIGHT OF TOPS					
	NEBRASKA NO. 10	NEBRASKA NO. 12	SCHMIDT'S WHITE CAP	NEBRASKA NO. 10	NEBRASKA NO. 12	SCHMIDT'S WHITE CAP			
(1)	(2) *per cent.*	(4) *per cent.*	(4) *per cent.*	(5) *per cent.*	(6) *per cent.*	(7) *per cent.*	(8) *cc.*	(9) *degrees C.*	(10) *per cent.*
March 4–21							24.8	23.4	56
March 22–April 11	8.74	8.68	10.44	11.25	11.45	14.86	23.7	21.3	48
April 12–18	7.66	5.83	7.31	9.70	8.06	8.55	17.7	20.0	53
April 19–25	5.64	7.45	5.64	7.23	7.86	8.32	18.3	20.4	59
Average	7.35	7.32	7.80	9.39	9.12	10.58			

[1] Daily rates of increase (r), in per cent., were calculated according to the BLACKMAN (2) formula, $\frac{\log_e W_1 - \log_e W_0}{t_2 - t_1} = r$, where W_0 and W_1 represent the dry weight of the plants at the beginning and end, respectively, of the experimental interval, $t_2 - t_1$, the time between each two successive harvests.

TABLE VI

RATES OF GROWTH AND ATMOSPHERIC CONDITIONS FOR CORN[1] GROWN IN THE GREENHOUSE, 1926, IN SAND CULTURES (SERIES 2)

PERIOD	DAILY RATE OF INCREASE IN LEAF AREA			DAILY RATE OF INCREASE IN DRY WT. OF TOPS			DAILY ATMOMETER LOSS	AVERAGE MEAN DAILY TEMPERATURES	RELATIVE HUMIDITY AT NOON
	NEBRASKA NO. 10	NEBRASKA NO. 12	F_1	NEBRASKA NO. 10	NEBRASKA NO. 12	F_1			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*cc.*	*degrees C.*	*per cent.*
March 31–April 9							23.2	21.5	51
April 10–16	16.93	19.84	19.26	19.33	20.57	20.55	27.8	22.4	32
April 17–24	13.57	11.50	14.91	12.60	11.16	15.81	29.1	23.0	38
April 25–30	8.25	11.96	10.63	15.07	20.49	17.70	19.8	20.5	47
May 1–7	10.04	9.83	7.27	12.36	12.45	9.86	27.4	22.5	38
May 8–14	9.78	3.28	5.41	12.07	8.56	10.37	30.3	22.1	40
Average	11.71	11.28	11.50	14.29	14.65	14.86			

[1] The F_1 is a cross between Nebraska 12 (♀) and Nebraska 10 (♂).

TABLE VII

RATES OF GROWTH AND ATMOSPHERIC CONDITIONS FOR CORN[1] GROWN IN THE FIELD, 1925 (SERIES 3)

PERIOD	DAILY RATE OF INCREASE IN LEAF AREA			DAILY RATE OF INCREASE IN DRY WEIGHT OF TOPS			TOTAL[2] RAINFALL FOR THE PERIOD	MEAN DAILY TEMPERATURE FOR THE PERIOD
	NEBRASKA NO. 10	NEBRASKA NO. 12	F_1	NEBRASKA NO. 10	NEBRASKA NO. 12	F_1		
(1)	(2) *per cent.*	(3) *per cent.*	(4) *per cent.*	(5) *per cent.*	(6) *per cent.*	(7) *per cent.*	(8) *inches*	(9) *degrees C.*
June 1–9	.....	.....	.....	.....	.....	.....	0.00	26.7
June 10–13	14.27	12.52	17.80	17.33	17.33	25.83	0.00	20.6
June 14–20	11.71	11.68	14.94	13.48	12.80	17.06	0.14	23.9
June 21–27	14.19	15.65	14.54	18.23	20.87	19.52	0.10	22.2
June 28–July 4	8.93	8.94	8.24	11.33	8.24	6.54	1.78	21.7
July 5–11	13.03	10.73	8.86	15.34	14.30	14.21	1.03	25.0
July 12–18	4.91	5.70	3.60	8.80	12.57	9.17	0.48	23.3
Average	11.17	10.87	11.33	14.08	14.35	15.39		

[1] The F_1 is a cross between Nebraska 12 (♀) and Nebraska 659 (♂).

[2] 0.68 inches precipitation for last three days of May.

longer than those of Nebraska 12 or the F_1 as the plants approached maturity, which may account in part for the greater final yield of tops and grain from Nebraska 10 than from Nebraska 12. In all 3 series, the differences in dry weights of the strains were more marked than the differences in leaf areas, thus indicating that the leaves of the different strains were probably functioning with different degrees of efficiency in the assimilation of carbon.

Growth rates

Data on the rates of growth and the atmospheric conditions for the 3 series are given in tables V, VI, and VII. The daily rates of increase in leaf area and in dry weight of tops were calculated according to Blackman's formula (**2**). In general, the daily rates of increase in leaf area varied in the same direction as daily rates of increase in dry weight of tops but the rates were not proportional nor equal.

In series 1, there was a steady decline in growth rates with the age of the plant in all three strains, which is in accord with the findings of other workers studying growth rates. In series 2 and 3, the general decline in growth rates, although apparent, was by no means steady, particularly for dry weight of tops. In series 2, the most notable deviation in daily rates of increase in dry weight of tops was for the period April 25–30 when the rates of all strains were higher than for the preceding period. Since this deviation was accompanied by a marked increase in relative humidity of the air, the change in humidity may be taken as the cause.

The daily rates of increase in dry weights of tops for series 3, were abnormally low during the periods June 14–20, and June 28–July 4. The depression in the first period may be attributed to the absence of rain from May 30 to June 16. During the latter part of the period soil moisture had been so depleted by evaporation and transpiration that it constituted a limiting factor in growth. Light rains coming between June 16 and 25 caused an increase in growth, but the precipitation was not sufficient to maintain growth rates in the June 28–July 4 period. After July 1, rainfall was adequate for normal growth.

It may be noted in each series that growth rates, as measured by increase in dry weight of tops, were not paralleled by corresponding increases in leaf area. The conditions which caused changes in the growth rates based on dry weight, apparently operated by modifying the efficiency with which a given area of leaf functioned, as well as by changing the total leaf area.

Moreover, the efficiency with which the leaves of the various strains functioned was apparently not equal. In general, the rates of increase of leaf area were greater for Nebraska 10 than for Nebraska 12, but the rates

TABLE VIII

PIGMENT FORMATION IN CORN GROWN IN SAND CULTURES IN THE GREENHOUSE, 1925, WITH RELATION TO THE AREA AND DRY WEIGHT OF THE LEAVES (SERIES 1)

AGE OF PLANTS	STRAIN USED	TOTAL LEAF AREA EXTRACTED	AMOUNT OF PIGMENT PER 100 SQ. CM. OF LEAF[1]						CALCULATED DRY WEIGHT OF FRESH LEAVES EXTRACTED	AMOUNT OF PIGMENT PER GRAM OF LEAF (DRY WEIGHT) EXTRACTED[1]					
			CHLOROPHYLL		CAROTIN		XANTHOPHYLL			CHLOROPHYLL		CAROTIN		XANTHOPHYLL	
			ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE		ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
days		*sq. cm.*	*mg.*		*mg.*		*mg.*		*gm.*	*mg.*		*mg.*		*mg.*	
17	Nebraska 10	284.8	2.61	100	0.038	100	0.018	100	0.128	22.7	100	0.335	100	0.158	100
17	Nebraska 12	340.0	1.57	60	0.048	124	0.012	67	0.308	31.3	138	0.527	157	0.140	89
12	Schmidt's White Cap	67.7	3.07	118	0.066	174	0.009	50	0.077	30.2	133	0.576	172	0.080	51
38	Nebraska 10	1035.6	4.06	156	0.125	329	0.112	622	2.196	19.2	84	0.590	176	0.529	335
38	Nebraska 12	1671.6	3.60	138	0.121	318	0.138	767	3.116	19.3	85	0.650	194	0.746	472
33	Schmidt's White Cap	386.7	2.46	94	.0163	429	0.244	1356	0.962	14.9	66	0.660	197	0.982	622
45	Nebraska 10	1644.4	3.07	118	.0104	274	0.108	600	4.844	10.4	46	0.348	104	0.366	232
45	Nebraska 12	2448.0	2.46	94	0.164	432	0.097	539	6.444	9.3	41	0.403	120	0.371	235
40	Schmidt's White Cap	688.1	2.87	110	0.119	313	0.054	300	2.096	9.4	41	0.391	117	0.181	115
Average	Nebraska 10		3.25		0.089		0.079			17.4		0.424		0.351	
	Nebraska 12		2.54		0.111		0.082			20.0		0.528		0.419	
	Schmidt's White Cap		2.80		0.116		0.102			18.2		0.542		0.414	

[1] Relative values are calculated using Nebraska 10 at 17 days as 100.

TABLE IX

PIGMENT FORMATION IN CORN GROWN IN SAND CULTURES IN THE GREENHOUSE, 1926, WITH RELATION TO THE AREA AND DRY WEIGHT OF THE LEAVES (SERIES 2)

AGE OF PLANTS	STRAIN USED[1]	TOTAL LEAF AREA EXTRACTED	AMOUNT OF PIGMENT PER 100 SQ. CM. OF LEAF[2]						CALCULATED DRY WEIGHT OF FRESH LEAVES EXTRACTED	AMOUNT OF PIGMENT PER GRAM (DRY WEIGHT) OF LEAF EXTRACTED[2]					
			CHLOROPHYLL		CAROTIN		XANTHOPHYLL			CHLOROPHYLL		CAROTIN		XANTHOPHYLL	
			ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE		ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
days		*sq. cm.*	*mg.*		*mg.*		*mg.*		*gm.*	*mg.*		*mg.*		*mg.*	
10	Nebraska 10	61.5	1.75	100	0.093	100	0.085	100	0.116	9.2	100	0.490	100	0.450	100
10	Nebraska 12	67.6	1.90	109	0.100	108	0.095	112	0.118	10.9	118	0.576	118	0.547	122
10	F_1	78.3	2.06	118	0.121	130	0.118	139	0.146	11.0	120	0.646	132	0.632	140
17	Nebraska 10	128.2	2.32	133	0.112	120	0.134	158	0.276	10.8	117	0.520	106	0.624	139
17	Nebraska 12	162.7	2.71	155	0.140	151	0.115	135	0.316	14.0	152	0.718	147	0.593	132
17	F_1	183.7	2.41	138	0.123	132	0.118	139	0.388	11.4	124	0.584	119	0.563	125
25	Nebraska 10	207.7	2.39	137	0.100	108	0.112	132	0.438	11.3	123	0.474	97	0.534	119
25	Nebraska 12	460.5	2.60	149	0.106	114	0.062	73	0.822	14.6	159	0.593	121	0.344	76
25	F_1	610.7	2.90	166	0.125	134	0.089	105	1.318	13.4	146	0.573	117	0.415	92
31	Nebraska 10	445.0	2.27	130	0.091	98	0.115	135	1.31	7.7	84	0.306	62	0.392	87
31	Nebraska 12	853.4	2.00	114	0.108	116	0.132	155	2.32	7.4	80	0.395	81	0.486	108
31	F_1	1126.0	2.72	155	0.132	142	0.138	162	3.31	9.2	100	0.452	92	0.470	104
38	Nebraska 10	655.0	2.66	152	0.106	114	0.161	189	2.13	8.2	89	0.323	66	0.498	111
38	Nebraska 12	1342.6	2.99	171	0.130	140	0.134	158	4.00	10.3	112	0.435	89	0.447	99
38	F_1	1417.2	2.89	165	0.134	144	0.143	168	4.72	8.6	93	0.404	82	0.427	95
45	Nebraska 10	1249.4	4.41	252	0.134	144	0.120	141	4.45	12.4	135	0.376	77	0.338	75
45	Nebraska 12	1926.8	5.58	319	0.164	176	0.101	119	6.71	16.0	174	0.472	96	0.291	65
45	F_1	2318.0	6.68	382	0.174	187	0.112	132	9.07	17.1	186	0.446	91	0.286	64
Average	Nebraska 10		2.63		0.106		0.121			9.9		0.415		0.473	
	Nebraska 12		2.96		0.125		0.106			12.2		0.531		0.451	
	F_1		3.28		0.135		0.120			11.8		0.517		0.465	

[1] The F_1 is a cross between Nebraska 12 (♀) and Nebraska 10 (♂).

[2] Relative values are calculated using Nebraska 10 at 10 days as 100.

TABLE X

TABLE X

PIGMENT FORMATION IN CORN GROWN IN THE FIELD, 1925, WITH RELATION TO THE AREA AND DRY WEIGHT OF THE LEAVES (SERIES 3)

AGE OF PLANTS	STRAIN USED[1]	TOTAL LEAF AREA EXTRACTED	AMOUNT OF PIGMENT PER 100 SQ. CM. OF LEAF[2]						CALCULATED DRY WEIGHT OF FRESH LEAVES EXTRACTED	AMOUNT OF PIGMENT PER GRAM (DRY WEIGHT) OF LEAF EXTRACTED[2]					
			CHLOROPHYLL		CAROTIN		XANTHOPHYLL			CHLOROPHYLL		CAROTIN		XANTHOPHYLL	
			ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE		ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
days		*sq. cm.*	*mg.*		*mg.*		*mg.*		*gm.*	*mg.*		*mg.*		*mg.*	
10	Nebraska 10	68.2	7.44	187	0.665	652	1.100	853	0.142	35.7	300	3.194	1044	5.284	1369
10	Nebraska 12	139.2	4.69	118	0.181	177	0.387	300	0.274	23.8	200	0.924	302	1.963	509
10	F_1	158.6	6.42	161	0.112	110	0.380	295	0.328	31.1	261	0.542	177	1.837	476
14	Nebraska 10	136.4	4.99	125	0.189	185	0.378	293	0.322	21.1	177	0.798	261	1.604	416
14	Nebraska 12	174.8	5.90	148	0.100	98	0.869	674	0.402	25.7	216	0.433	142	3.779	979
14	F_1	338.8	5.40	136	0.019	19	0.028	22	0.960	19.0	160	0.066	22	0.100	26
21	Nebraska 10	171.2	.....	.....	0.047	46	0.209	162	0.438	.....	.....	0.181	59	0.815	211
21	Nebraska 12	378.0	3.67	92	0.083	81	0.297	230	0.898	15.5	130	0.350	114	1.247	323
21	F_1	737.6	5.17	130	0.129	126	0.169	131	2.296	16.6	140	0.410	134	0.541	140
28	Nebraska 10	315.8	3.98	100	0.102	100	0.129	100	1.060	11.9	100	0.306	100	0.386	100
28	Nebraska 12	540.0	4.54	114	0.132	129	0.149	116	1.650	14.9	125	0.433	142	0.486	126
28	F_1	211.5	7.27	183	0.219	214	0.154	119	0.790	19.5	164	0.586	192	0.410	106
35	Nebraska 10	546.6	5.78	145	0.115	113	0.143	111	1.960	16.1	135	0.321	105	0.398	103
35	Nebraska 12	826.8	5.44	137	0.132	129	0.149	116	2.580	17.4	146	0.425	139	0.480	124
35	F_1	1441.0	5.86	147	0.136	133	0.109	84	5.270	16.0	134	0.370	121	0.300	78
42	Nebraska 10	1164.4	4.60	116	0.138	135	0.181	140	5.090	10.5	88	0.318	104	0.417	108
42	Nebraska 12	1484.8	3.61	91	0.153	150	0.160	124	5.350	10.0	84	0.423	138	0.444	115
42	F_1	2214.6	4.03	101	0.204	200	0.148	115	9.320	9.6	81	0.486	159	0.352	91
49	Nebraska 10	1493.8	4.27	107	0.183	179	0.211	164	7.560	8.4	71	0.361	118	0.417	108
49	Nebraska 12	2064.0	4.90	123	0.193	189	0.169	131	9.750	10.4	87	0.408	133	0.360	93
49	F_1	2682.4	5.62	141	0.215	211	0.175	136	13.610	11.1	93	0.423	138	0.346	90
Average last 4 harvests	Nebraska 10		4.66		0.134		0.166			11.7		0.326		0.404	
	Nebraska 12		4.62		0.152		0.157			13.2		0.422		0.440	
	F_1		5.69		0.193		0.146			14.0		0.466		0.352	

[1] The F_1 is a cross between Nebraska 12 (♀) and Nebraska 659 (♂).

[2] Relative values are calculated using Nebraska 10 at 28 days as 100.

of increase in dry weight averaged higher for Nebraska 12 than Nebraska 10. Also, the average rates of increase in dry weight of the F_1 crosses were higher than those of Nebraska 10 in series 2 and 3, although the average rates of increase in leaf area were about the same.

Chloroplast pigment content per unit of leaf

Data on the pigment content of each strain per unit of leaf area, and of leaf weight, at successive harvests of the 3 series are given in tables VIII, IX, and X. It has been noted that the leaves of the various strains apparently functioned with varying efficiencies which could not be explained on the basis of differences in leaf area. Since chlorophyll is known to be a primary agent in the synthesis of plant food, it is possible that the variation in concentration of this pigment is a contributing factor in the rate of carbon assimilation, and therefore in growth. The chloroplast pigment concentrations have been expressed both on the basis of unit of leaf area, and of unit of dry weight, because the two criteria give quite different values.

In series 1, on the basis of milligrams of pigment per 100 square cm. of leaf, there was no distinct trend noted in chlorophyll concentration at successive harvests; also, the relation between these concentrations and the growth rates of a single strain was not consistent. On the basis of chlorophyll concentration per gram of leaf, the highest values for each strain were found in the earlier harvest, with later harvests showing declining values. These declining values were associated with increases in weight of leaf per unit of area at successive harvests. The inverse relation between chlorophyll content per gram of leaf and age of the plant was also observed in series 3. Since there was no decline noted in chlorophyll concentration per unit of leaf area, it seems clear that this pigment was much more closely correlated with the area of leaves than with their dry weight. Unlike the other series, the plants of series 2 showed no consistent decline in chlorophyll per gram of leaf at the successive harvests. This discrepancy is unintelligible at this time because too little is known about the effects of environmental conditions on pigment formation.

There was only one period, occurring in series 3, when there seemed to be a close correlation between growth rates and the chlorophyll concentrations of all strains. The concentrations of the pigment on either the dry weight or leaf area basis, were somewhat higher at the beginning of the period July 5–11 than for the preceding one. This increase in pigment concentration was accompanied by an improvement in the supply of soil moisture and appears to have been caused by it.

In series 1, it may be noted that correlation between concentrations of chlorophyll and increase in dry weight of the various strains was fairly good. Nebraska 12 had the lowest concentration per unit of area and the lowest

growth rate. Nebraska 10 was intermediate in concentration of chlorophyll and in growth, while Schmidt's White Cap has the highest average values for both characters.

The data from series 2 are of the most value in showing the relation between chlorophyll concentration of the strains and the rates of increase in dry weight of their tops, because the environmental conditions were more nearly uniform throughout the entire period than for the other two series. Nebraska 10, which had the lowest average rate of growth, showed the lowest values of chlorophyll per unit of leaf area and per unit of dry weight in five out of six harvests. Nebraska 12 which was intermediate in the average increase in dry weight of tops, had intermediate values of chlorophyll per unit of leaf area four times out of six, and on the basis of unit leaf weight showed intermediate values twice, the highest values three times, and the lowest once. The F_1 plants of this series which had the highest average rate of increase in dry weight of tops also had the highest concentration of chlorophyll per unit of area five times out of six, and had the highest values per gram of leaf three times, intermediate values twice, and lowest value once.

The chlorophyll concentrations per unit area of leaf in series 3 were somewhat erratic for the first three harvests, probably because of high temperatures and lack of rainfall. With these harvests eliminated, the last four harvests showed a fairly close relation between chlorophyll content of the various strains and the average rates of increase in dry weight of tops. Nebraska 10, with the lowest average growth rate, had the lowest set of concentration values for chlorophyll, Nebraska 12 showed intermediate values in both rate of growth and chlorophyll concentration, and the F_1 had the highest values for both characters.

A significant feature of the chlorophyll concentrations per unit of leaf area was the relatively high values observed in all three strains of series 3, as compared with the first two series. The explanation may lie in the light relations of the three series. The plants of series 3 were grown during a season when the days were longer and the light more intense than those of series 1 and 2. Also, being grown in the field, no portion of the spectrum was excluded or diminished in intensity by glass. Unfortunately no exact measures of light conditions were made in connection with these experiments.

When the concentrations of carotin and chlorophyll are compared for all three series, it becomes apparent that there was a fairly close correlation between the concentrations of the two pigments. The correlations were somewhat higher on the basis of unit dry weight than on the basis of unit leaf area. Apparently, conditions which affect the concentration of one pigment also affect that of the other in like manner. The most notable

exception to this condition occurred at the first two harvests of series 3. Carotin values were very high, especially on the dry weight basis, and their abnormal values were not accompanied by similar values of chlorophyll. These periods were characterized by unusually high temperatures and lack of rainfall. The different strains did not behave alike under these conditions; Nebraska 10 had a much higher concentration of carotin than Nebraska 12, while the F_1 values were apparently quite normal. These variations did not seem to be significantly related to the growth rates for the periods following the analyses.

With the exception of series 1, the relation between rates of increase in dry weight of tops and concentrations of carotin was even closer than the relation between the growth rates and chlorophyll concentration of the various strains. In series 1, Nebraska 10 averaged lower concentrations of carotin on both the dry weight and leaf area bases than did Nebraska 12 but had slightly higher average growth rates. The concentrations of carotin in Schmidt's White Cap were greater than those of either Nebraska 10 or Nebraska 12 and these were accompanied by higher growth rates.

In series 2, the correlation between concentrations of carotin per unit of leaf area and the rates of growth of the various strains was very good. Nebraska 10 had the lowest set of concentrations and the lowest average growth rate, Nebraska 12 gave intermediate values and the F_1 the highest values. On the basis of carotin concentration per gram of leaf, Nebraska 10 again had the lowest average values for both pigment concentration and growth rate, but Nebraska 12 had a higher pigment concentration than the F_1 and a lower average rate of growth.

Disregarding the few abnormal data of the first harvests of series 3, there was a very good correlation between the average carotin concentration of the three strains and their average growth rates. The relation was slightly closer on the basis of unit leaf area than on the basis of unit dry weight; but Nebraska 10 had the lowest average concentrations of carotin and also the lowest average calculated on either basis; Nebraska 12 gave intermediate concentrations, and the F_1 gave highest.

The concentrations of xanthophyll fluctuated so widely in these series that it is difficult to detect any distinct trends or relationships. The concentrations of xanthophyll in plants of Nebraska 10 were not consistently higher or lower than those of the Nebraska 12 plants on either the basis of unit leaf area or of unit dry weight. Xanthophyll concentrations resembled carotin concentrations only during the first two harvests of series 3 when high concentrations of both pigments occurred in conjunction with high temperatures and a scanty moisture supply.

Neither carotin nor xanthophyll exhibited any decline in concentration per gram of leaf with successive harvests, such as was observed with chloro-

TABLE XI

TOTAL CHLOROPLAST PIGMENTS FOUND IN THE LEAF BLADES OF CORN PLANTS GROWN IN SAND CULTURES IN THE GREENHOUSE, 1925 (SERIES 1)

AGE OF PLANTS	TOTAL CHLOROPHYLL, AVERAGE PER PLANT			TOTAL CAROTIN, AVERAGE PER PLANT			TOTAL XANTHOPHYLL, AVERAGE PER PLANT		
	NEBRASKA 10	NEBRASKA 12	SCHMIDT'S WHITE CAP[1]	NEBRASKA 10	NEBRASKA 12	SCHMIDT'S WHITE CAP	NEBRASKA 10	NEBRASKA 12	SCHMIDT'S WHITE CAP
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
days	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
17	1.64	1.42	3.32	0.024	0.042	0.071	0.011	0.011	0.010
38	16.28	20.11	23.83	0.501	0.676	1.579	0.449	0.771	2.364
45	21.02	20.67	46.38	0.712	1.378	1.923	0.739	0.815	0.873

[1] Schmidt's White Cap was 5 days younger throughout the series than indicated.

TABLE XII

TOTAL CHLOROPLAST PIGMENTS FOUND IN LEAF BLADES OF CORN PLANTS GROWN IN THE GREENHOUSE IN 1926 (SERIES 2)

AGE OF PLANT	TOTAL CHLOROPHYLL, AVERAGE PER PLANT			TOTAL CAROTIN, AVERAGE PER PLANT			TOTAL XANTHOPHYLL, AVERAGE PER PLANT		
	NEBRASKA 10	NEBRASKA 12	F_1[1]	NEBRASKA 10	NEBRASKA 12	F_1[1]	NEBRASKA 10	NEBRASKA 12	F_1[1]
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
days	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
10	0.34	0.56	0.77	0.018	0.029	0.045	0.016	0.028	0.044
17	1.47	3.19	3.47	0.071	0.165	0.177	0.085	0.135	0.170
25	4.50	7.67	13.78	0.188	0.313	0.594	0.211	0.183	0.423
31	7.00	12.09	24.46	0.281	0.653	1.187	0.354	0.798	1.241
38	16.54	35.97	43.22	0.659	1.564	2.004	1.001	1.612	2.138
45	54.41	93.38	145.58	1.653	2.744	3.792	1.480	1.690	2.441

[1] The F_1 in this series was a cross between Nebraska 12 (♀) and Nebraska 10 (♂).

TABLE XIII

TOTAL CHLOROPLAST PIGMENTS FOUND IN THE LEAF BLADES OF CORN PLANTS GROWN IN THE FIELD DURING THE SUMMER OF 1925 (SERIES 3)

AGE OF PLANT	TOTAL CHLOROPHYLL, AVERAGE PER PLANT			TOTAL CAROTIN, AVERAGE PER PLANT			TOTAL XANTHOPHYLL, AVERAGE PER PLANT		
	NEBRASKA 10	NEBRASKA 12	F_1[1]	NEBRASKA 10	NEBRASKA 12	F_1[1]	NEBRASKA 10	NEBRASKA 12	F_1[1]
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
days	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
10	3.10	3.10	6.24	0.277	0.120	0.109	0.459	0.256	0.369
14	3.68	6.44	10.70	0.139	0.109	0.038	0.279	0.949	0.055
21	.....	9.09	29.15	0.079	0.206	0.727	0.350	0.735	0.953
28	18.01	33.61	113.40	0.462	0.977	3.416	0.584	1.103	2.402
35	48.56	75.25	162.54	0.966	1.826	3.772	1.201	2.061	3.023
42	96.19	105.95	208.14	2.886	4.490	10.536	3.785	4.696	7.644
49	125.70	214.37	373.78	5.387	8.444	14.299	6.211	7.394	11.639

[1] The F_1 in this series was a cross between Nebraska 12 (♀) and Nebraska 659 (♂).

phyll. On the basis of unit leaf areas, there was a slight increase in concentrations of carotin with successive harvests in all three series, provided the first two harvests of series 3 are disregarded. These data indicate that the formation of the carotinoids was closely related to the weight of the leaves, whereas the formation of chlorophyll was closely related to their surface area. Evidently carotin and xanthophyll have somewhat similar modes of formation and perhaps have similar physiological functions.

Total chloroplast pigment content and yields of dry matter

The total chloroplast pigment content in the leaf blades of a plant is dependent upon the concentration of pigment per unit of area and the total area of the leaves. If the chloroplast pigments are primary agents in the synthesis of organic compounds in the plant, there should be a fairly close correlation between the total amount of these pigments present and the total dry weight produced. The data presented above indicate only the quantities of chloroplast pigments found in the leaf blades, ignoring that small fraction which was present in the leaf sheaths and stems; and the data on dry weights do not include roots. If correlations between quantities of chloroplast pigments in leaf blades and dry weight of tops are high, similar correlations may reasonably be expected between the total amount of such pigments in the whole plant and total dry weight, providing the ratios of tops to roots are similar for all strains. The total quantity of chloroplast pigments found in the leaf blades of the strains in these experiments is given in tables XI, XII, and XIII.

There was a well-defined correlation between the total chlorophyll content of the various strains and their respective dry weight of tops. (Compare tables II, III and IV with tables XI, XII and XIII.) Nebraska 10 had the smallest quantity of chlorophyll per plant at every harvest of series 2 and 3, and also had the smallest dry weight of tops at all times. Nebraska 12 had larger quantities of chlorophyll and greater dry weights of tops than Nebraska 10 for every harvest in series 2 and 3, but less than Schmidt's White Cap in series 1 and the F_1 crosses in series 2 and 3.

The correlation between the total carotin content of leaves of the various strains and their total dry weight of tops was nearly as close as that noted for chlorophyll, with the exception of the first two harvests of series 3. In series 2 and 3, Nebraska 10 had the lowest carotin content and the lowest dry weight of tops, and Nebraska 12 had higher values for both characters than Nebraska 10, but lower values than the F_1 crosses.

Total xanthophyll values were also closely correlated with dry weight of tops, but not as closely as carotin values. In addition to the unusual values obtained in the first two harvests of series 3, the values for the first and third harvests of series 1 and the third harvest of series 2 did not show

TABLE XIV

THE RELATIONS BETWEEN THE DRY WEIGHT OF CORN PLANTS (TOPS) AND THE TOTAL CONTENT OF CHLOROPLAST PIGMENTS IN THE LEAF BLADES AS SHOWN BY RATIOS (SERIES 2)

AGE OF PLANTS	RATIO OF DRY WEIGHT TO TOTAL CHLOROPHYLL CONTENT			RATIO OF DRY WEIGHT TO TOTAL CAROTIN CONTENT			RATIO OF DRY WEIGHT TO TOTAL XANTHOPHYLL CONTENT		
	NEBRASKA 10	NEBRASKA 12	F_1	NEBRASKA 10	NEBRASKA 12	F_1	NEBRASKA 10	NEBRASKA 12	F_1
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
days									
10	179.4	167.9	158.4	3389	3241	2711	3812	3357	2773
17	160.5	124.5	148.1	3324	2406	2904	2776	2941	3024
25	144.8	126.5	132.1	3441	3099	3066	3066	5301	4305
31	228.6	274.6	215.5	5694	5084	4440	4520	4160	4247
38	229.7	220.7	255.4	5766	5077	5509	3796	4925	5164
45	161.9	154.6	156.8	5330	5262	6021	5953	8544	9353
Average	184.1	178.1	177.7	4491	4028	4108	3987	4871	4811

the same differences between strains as their dry weights. With these exceptions, Nebraska 10 was lowest in total xanthophyll and dry weight of tops, and Nebraska 12 was higher than Nebraska 10 but lower than Schmidt's White Cap and the F_1 crosses.

It is important also to determine the degree of relationship between the quantity of each chloroplast pigment and dry weight of tops. The data are too few to permit the calculation of reliable coefficients of correlation but the relation may be expressed in another fashion. Series 2 is most satisfactory for a study of this sort, since the environmental conditions were more uniform throughout the entire experimental period than for the other series. The relation has been expressed in table XIV in terms of ratios between grams of dry matter and grams of pigment.

The average ratios between dry weight and chlorophyll content were very similar for all three strains of corn. A rise or fall in the ratio of one strain at any harvest was usually accompanied by a similar rise or fall in the other strains. The fluctuations in ratios for the various harvests signify that total chlorophyll content was not the only causal factor involved in growth, but the relatively small size of the fluctuations indicates that it was a very important factor.

The average ratios between dry weight and carotin content were similar for all three strains of corn, but displayed greater differences than those noted for chlorophyll. The differences between ratios of the strains at various harvests were much more marked than those for chlorophyll. In general, however, there was a distinct tendency for fluctuations of all three strains to move in the same direction.

The average ratios (and those for each harvest) between dry weight and xanthophyll content were even less consistent than those noted for carotin. In only two of the six harvests did the ratios of all three strains fluctuate in the same direction.

It seems clear from these data that a close relation existed between total chlorophyll content and dry weight of tops for all three strains of corn, at the various stages of growth considered. It may be assumed that the relation was a causal one since chlorophyll is known to be essential in photosynthesis. The physiological functions of carotin and xanthophyll are not known, and the correlations between quantities of these pigments and dry weights in this series of plants have not demonstrated their essentiality in growth. The data in table XIV indicate that the closest correlation existed between dry weight and chlorophyll, the next in order between dry weight and carotin, and the lowest correlations between dry weight and xanthophyll.

Discussion and conclusions

Although the question of the relations between chloroplast pigments, leaf area, and dry weight has not been definitely defined by the data here

presented, certain points seem fairly clear. As a whole there is a much higher correlation between chlorophyll content and leaf area than between chlorophyll content and dry weight of leaves. On the other hand, data from series 2 and 3 indicate that carotin and xanthophyll are somewhat more closely related to leaf weight than to leaf area.

Chlorophyll concentrations per unit of area, or of dry weight, were not closely paralleled by rates of increase in dry weight of tops from period to period for a single strain, but chlorophyll concentrations of the various strains were rather closely correlated with their respective rates of increase in dry weight. Thus, the strain which had the lowest set of chlorophyll values per unit of leaf area, also had the lowest average rate of increase in dry matter, and *vice versa*. Likewise, the correlations between carotin concentrations and rates of increase in dry matter were fairly close, but the xanthophyll readings were so variable that no definite relation could be observed. These conclusions rest on the assumption that the leaves selected for pigment extraction were representative of all of the leaves on the plant with respect to pigment formation, leaf area and dry weight. Since comparable leaves from each plant of every strain of corn were harvested for each set of determinations, it seems reasonable to suppose that no great error has been introduced.

It has been suggested by some investigators that chlorophyll is being constantly synthesized and broken down in the leaf. If this is the case, the amount present at any one time in the leaf represents the result of an equilibrium between the synthetic and destructive processes. On this basis it is obvious that the chlorophyll content of a leaf or of a plant at any given instant may not be representative of that present at any other time during a weekly period. With constant fluctuations in quantity of chlorophyll as a result of environmental or cultural conditions, there is considerable chance of obtaining values for the pigment which do not represent average conditions and this may partly account for the failure to obtain perfect correlations between chlorophyll concentration and rates of increase in dry weight. The same observation applies also to measurements of carotin and xanthophyll. The data presented have not revealed how environmental or cultural conditions affect the chloroplast pigment content of a plant at any given time.

Internal factors not directly associated with pigment content are undoubtedly contributing factors in dry weight production. With adequate amounts of chlorophyll and carotinoids present, there still may be distinctly different rates of growth in different strains due to the effect of these undetermined internal factors.

Another significant point noted in these experiments was the close correlation between total chloroplast pigment content of the leaves of the

various strains and their respective dry weights. With but two exceptions, occurring in series 1, the strain having the highest chlorophyll content at any stage of development also had the highest dry weight of tops, and *vice versa*. The correlation was particularly clear in series 2 grown under uniform environmental conditions as far as this was possible. Since chlorophyll has been shown to be essential for photosynthesis, and since these data show a high correlation between total chlorophyll content and dry weight, the logical conclusion is that superiority in dry weight of the plants of one strain over those of another strain similarly treated was largely due to superiority in chlorophyll content. Undoubtedly other factors are involved since the ratio between grams of dry matter and grams of chlorophyll was not absolutely constant; but these factors must have been of minor value, or the average ratios of the three strains of corn would not have been so nearly alike.

Since none of these strains could be classed as chlorophyll deficient by observation, it seems likely that chlorophyll tests may become of considerable value in the improvement of corn. If the more vigorous strains may be selected on this basis plus the visual evidences of vigor, a portion of the tedious hybridizing and progeny testing which is now necessary for selection of desirable strains may be eliminated.

Although a fairly close correlation appears to exist between dry weight and total quantities of carotin and xanthophyll, it is not safe to assume that the relation is causal because these pigments have not been shown to be essential for synthesis of organic matter. Their occurrence in amounts rather uniformly proportional to chlorophyll in series 2 suggests a similar relation to dry weight but the wide fluctuations which occurred under field conditions also suggest that the carotinoids may play a different rôle in the life processes of the plant from that usually attributed to chlorophyll.

Summary

Data on leaf area, dry weight of tops and chloroplast pigment content were obtained from three series of corn plants. Two of the series were grown in the greenhouse, using sand cultures with culture solutions added by the constant drip method. The other series was grown in the field. Three strains of corn were grown in each experiment and two of these strains were used in all three series, namely, Nebraska 10 and Nebraska 12, which have been self-pollinated for 17 generations. The other strain used was either a commercial variety or an F_1 cross of two selfed strains. Weekly harvests and determinations of dry weight of tops, leaf area (expanse), and chloroplast pigment content were made. Growth rates were calculated for both dry weight of tops and leaf area, and data on atmospheric conditions for the same periods were obtained. The pigments

were extracted from comparable leaves of the different plants, separated, purified, and made up to a definite volume for colorimetric determinations in a Duboscq colorimeter. Artificial color standards were used in measuring the various pigments, each color standard having been evaluated in terms of milligrams of the pigment to be measured.

1. Rates of increase of leaf area and of dry weight of tops both declined normally as the plant aged, but they were not always closely correlated.

2. The strain or variety having the smallest leaf area during the period of an experiment also produced the lowest dry weight of top, and *vice versa*.

3. Chloroplast pigment measurements failed to show a proportional relation to the measured growth rates of a given strain, no matter whether expressed on the basis of pigment per 100 sq. cm. of leaf area or per gram of leaf (dry weight).

4. The strain which showed high chlorophyll and carotin concentrations per unit of leaf-area also had high average rates of increase in dry weight of tops, and *vice versa*. Xanthophyll values were variable and showed no definite relation to rates of growth.

5. Chlorophyll was more closely correlated with leaf area than with dry weight of leaves. Carotin and xanthophyll, however, were not as closely correlated with leaf as with dry weight of leaves.

6. The dry weights per unit of leaf area increased for all strains of corn at successive harvests. Nebraska 12 leaves had the lowest weight per 100 sq. cm. of leaf area in all three series, Nebraska 10 next lowest, and the commercial variety or F_1 cross, the highest weight.

7. In two of the three series of plants grown, the weight of chlorophyll per gram of leaf (dry weight) decreased as the leaves increased in weight per 100 sq. cm. of area. In the other series no clear relation could be observed in this respect.

8. There was a close correlation between the total quantity of chlorophyll contained in the leaves of the various strains of maize and their dry weight of tops at successive harvests. Correlations between total carotin content and dry weight, and between total xanthophyll content and dry weight were close enough to be significant but were not as exact as those noted between total chlorophyll content and dry weight.

9. The average ratios between total chlorophyll and dry weight of tops were practically identical with all three strains of corn in series 2. Ratios between dry weight and carotin content of the same series were more variable than those noted between dry weight and chlorophyll but a certain constancy was apparent. Ratios between dry weight and xanthophyll are so variable that their significance is doubtful.

LABORATORY OF PLANT PHYSIOLOGY,
NEW JERSEY AGRICULTURAL EXPERIMENT STATION.

LITERATURE CITED

1. Allison, R. V. The relation of aeration to the development of the soybean plant in artificial culture. New Jersey Agr. Exp. Sta. Ann. Rpt. pp. 338–344. 1921.
2. Blackman, V. H. The compound interest law and plant growth. Ann. Bot. **33**: 353–360. 1919.
3. Jones, L. H., and Shive, J. W. Effect of ammonium sulfate upon plants in nutrient solutions supplied with ferric phosphate and ferrous sulfate as sources of iron. Jour. Agr. Res. **21**: 701–728. 1921.
4. Montgomery, E. G. Correlation studies in corn. Nebraska Agr. Exp. Sta. 24th Ann. Rpt. 113, 114. 1911.
5. Schertz, F. M. The quantitative determination of carotin by means of the spectrophotometer and the colorimeter. Jour. Agr. Res. **26**: 383–400. 1923.
6. ————————. The quantitative determination of xanthophyll by means of the spectrophotometer and the colorimeter. Jour. Agr. Res. **30**: 253–261. 1925.
7. ————————. The extraction and separation of chlorophyll (α and β), carotin and xanthophyll in fresh green leaves, preliminary to their quantitative determination. Plant Phys. **3**: 211–216. 1928.
8. Shive, J. W. A study of physiological balance in nutrient media. Physiol. Res. **1**: 373–397. 1915.
9. ————————, and Stahl, A. L. Constant rates of continuous solution renewal for plants in water cultures. Bot. Gaz. **84**. 317–323. 1927.
10. Spoehr, H. A. Photosynthesis. New York. 1926.
11. Sprague, H. B. Preliminary report on the relations between pigment formation, leaf area, and dry weight of corn grown in sand cultures. Ann. Rpt. New Jersey Agr. Exp. Sta. for the year ending June 30, 1925. pp. 332–337. 1925.
12. Stiles, Walter. Photosynthesis. The assimilation of carbon by green plants. London. 1925.
13. Tottingham, W. E. A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. Res. **1**: 133–245. 1914.
14. Willstätter, R., and Stoll, A. Untersuchungen über Chlorophyll. Berlin. 1913.
15. ————————, ————————. Untersuchungen über die Kohlensäureassimilation. Berlin. 1918.

THE TOXIC SUBSTANCE PRODUCED BY THE EYE-SPOT FUNGUS OF SUGAR CANE, *HELMINTHOSPORIUM SACCHARI* BUTLER

ATHERTON LEE

(WITH TWO FIGURES)

Introduction

Eye spot is the name given to a leaf disease of sugar cane caused by the fungus *Helminthosporium* (*Cercospora*) *sacchari* Butler.[1] The disease is characterized by small spots on the leaves of most varieties, but on more susceptible varieties long streaks, several feet in length and sometimes 5/16ths of an inch in width, may result from infection. When there are many infections per leaf on such susceptible varieties, whole leaves may die, and with an inadequate leaf supply the growing point of the cane frequently dies also; the whole cane stalk then may rot from secondary infections or in other ways be useless for milling. In case the environmental conditions are very favorable for the disease, from 20 to 80 per cent. of the stalks may be killed in this manner, resulting in severe financial loss.

It has been carefully shown by LEE and MARTIN (**3**) and MARTIN and LEE (**4**) that nitrogen fertilizers greatly increase the severity of this disease. This increased severity was at first thought to result merely from more succulent, actively growing host tissues which resulted from the fertilizer applications, since it is usually found that eye spot is more severe on the young, new, actively growing leaves, while more slowly growing hardened leaves are not severely affected. However, as against this theory was the finding by LEE and MARTIN (**3**), that increased cane growth as a response to phosphoric acid, did not increase the severity of eye spot.

The nature of the long streaks resulting from *Helminthosporium* infection, although not a matter of apparent immediate commercial or economic importance, was a matter of curiosity to all concerned with the problems of eye-spot control. LARSEN (**2**) had shown very conclusively, at the time of carrying through the rules of proof connecting this *Helminthosporium* (*Cercospora*) with eye spot of sugar cane, that although the fungus could be isolated and re-isolated from the area within a centimeter or so of the point of infection, it could not be recovered from the long streaks which subsequently elongated from the point of infection.

[1] The identity of *Helminthosporium sacchari* Butler with *Cercospora sacchari* Van Breda de Haan, is a matter solely of assumption by cane pathologists; the question of identity has never been carefully examined although such identity seems probable.

Experimental results

Experiment I

Interruption of flow of nutrients not the cause of leaf streaks.—The theory was first advanced that the fungus after gaining entrance to the leaf kills a small area of the host tissue which is in immediate contact with the fungus hyphae, and that this killed area then shuts off the flow of nutrients and water up and down the leaf. From this interference result the streaks which emanate from the original point of infection. To prove or disprove this theory ten leaves of the sugar cane variety Hawaii 109, a very susceptible variety, were selected, and with a sharp sterile scalpel incisions from 0.5–1.5 cm. in length were made transversely across the leaves, thus cutting the vascular systems of the parallel-veined cane leaves and interrupting the flow of nutrients. All such incisions were without effect, and gave no indications of killing or streaks whatsoever. The experiment was repeated with identical results. These results indicated that the streaks resulting from *Helminthosporium* infection were not the result of an interruption of the nutrient supply.

The direction of the investigation then turned rather naturally to the possibility of the production of toxic substances by the eye-spot fungus, *Helminthosporium sacchari*.

Experiment II

Tests for toxic substances in filtrates of cultures in pure sucrose solution.—An attempt was then undertaken to determine the presence of a toxic substance in cultures of *H. sacchari*. The first attempts were with cultures of the fungus in solutions of 10 per cent. pure sucrose in water, autoclaved, in 500-cc. Erlenmeyer flasks. Growth in this medium was not vigorous; on the contrary it was rather sparse. At the end of 30 days the cultures were passed through Chamberland filters which separated the fungus from the culture solutions. Flasks of the filtrate were held for 10 days after the filtration and showed their sterility by absence of growth, either of *H. sacchari* or contaminating organisms. If a toxic substance had been produced by the fungus in the cultures it was expected that it would diffuse into the culture solutions, would pass through the filters while the fungus was withheld, and would evidence itself when inoculated into leaves of a susceptible cane variety. However, on inoculation of such filtrate into both young and old leaves of sugar cane, entirely negative results were secured.

In this and subsequent inoculation work both hypodermic needles and capillary tubing were used to introduce the filtrate into the leaves. The capillary tubing when filled with the filtrate was made to puncture the cells of the leaves and allowed to remain thus during the period of the experi-

ment. Unless otherwise noted, all tests for the toxic substance were made upon leaves of the very susceptible sugar-cane variety, H 109, the H standing for Hawaii, this variety being a seedling developed by the cane breeders of the Experiment Station of the Hawaiian Sugar Planters' Association.

Thinking that the toxic substance might not be in sufficient concentration, the filtrate was placed in desiccators over $CaCl_2$, and reduced to approximately one-fifth of its original volume, consequently increased to five times its original concentration. Such concentrated portions of the filtrate when inoculated into leaves of H 109 also gave entirely negative results.

Experiment III

Attempt to determine the presence of a toxic substance in filtrates of cultures in nutrient beef bouillon.—Thinking that the negative results with filtrates of cultures of *H. sacchari* in pure sucrose solutions might be due to the sparse growth of the fungus and sparse formation of a toxic substance, the experiment was repeated with cultures in nutrient beef-extract bouillon. The fungus was grown for 50 days in such cultures, then separated from the culture solutions by the use of Chamberland filters and the filtrates then concentrated to one-tenth their original volume over $CaCl_2$ in desiccator jars. The absence of *H. sacchari* and contaminating organisms was shown by holding the filtrate in sterile flasks for 10 days.

This concentrated filtrate was then inoculated into leaves of the H 109 cane variety. Then inoculations were made into young actively growing leaves and ten inoculations were made into older, more hardened mature leaves. After thirty days all inoculations but two were definitely negative. The two possibly positive results showed small red streaks, 1–2 mm. in width, one but 5 mm. in length and the other 24 mm. in length. On the basis of these results it could not possibly be concluded that a strong toxin was formed in the nutrient-bouillon cultures. At this stage of the investigation, the possibility that a toxic substance produced by *H. sacchari* was the cause of the leaf stripes of eye spot, appeared rather improbable.

Experiment IV

To determine the presence of a toxic substance in cultures in Richard's solution.—At this time the writer was re-reading the work by Brandes (**1**) on banana wilt and noted the use by Brandes of Richard's solution; this solution contains 10 gm. KNO_3, 5 gm. KH_2PO_4, 2.5 gm. $MgSO_4$, 20 mg. $FeCl_3$, and 50 gm. sucrose, in 1000 cc. of distilled water. The eye-spot fungus was grown in pure culture in flasks of this solution for 50 days. The cultures were then passed through a Chamberland filter, the culture solutions and diffused products of the fungus passing into the

filtrate and the fungus being separated out. The filtrate was a dark-brown clear liquid. Held in sterile flasks for 10 days there was no indication of growth of the *Helminthosporium* or contaminating organisms.

The project being rather discouraging at this point, no effort was made to concentrate the filtrate. And instead of inoculating the filtrate into the leaves by means of a hypodermic needle, or capillary tubing, 5 leaves of the H 109 variety were placed with their freshly cut ends immersed in the culture filtrate in sterile flasks. Identical H 109 leaves were placed in Richard's solution which had not been cultured with the *Helminthosporium*. This was done on May 31st, 1927, at 11:30 A. M. On the same date at 9:00 P. M. the leaves in the filtrate had begun to wilt and in 24 hours the wilt was clear-cut as compared to the control leaves in the uncultured medium. The leaves in the filtrate also showed long, reddish-brown streaks running longitudinally up the leaf similar in color and appearance to the streaks formed in natural cases of eye spot. The tentative conclusions from this experiment quoted directly from the notebook were:

Tentative conclusions.—(1) One would conclude from these preliminary results that a soluble toxic substance is produced by the eye-spot fungus which diffuses out from the fungus into the culture solution; and this toxic substance can be separated from the fungus and, independently of the fungus, produce on H 109 leaves the streak characteristic of eye spot.

(2) From the failures with cultures in pure sucrose solution and in beef bouillon this toxic substance does not seem to be formed in the absence of inorganic nitrogen.

(3) The toxic substance seems to be buffered in the presence of proteins.

On June 2nd, the same experiment was set up using a new set of flasks of the same kind of filtrate made on May 31st.

Experiment V

(1) To corroborate the results obtained in experiment iv and (2) to determine the effect of the toxic substance on leaves of the resistant yellow caledonia variety.—A 50-day old culture of the eye-spot fungus in Richard's solution was run through a Chamberland filter on May 30th. The filtrate was kept sterile for subsequent use.

On June 2nd, five small wide-mouthed straight-sided bottles of a diameter of 1.5 inches were filled to a height of 1.5 inches with this filtrate. Young freshly cut leaves of H 109 were placed with their butts immersed in the filtrate, one leaf in each bottle; these leaves were, in order of their age, the youngest leaf of the cane just emerged from the central spindle, the leaf lower than the youngest leaf, the next older leaf, the next, and the fifth leaf from the central spindle. In other words five leaves were taken

in order of their age from a stalk of H 109, and their butts immersed in the filtrate of the eye-spot culture, promptly after being cut.

Five identical bottles were filled to the same height, with Richard's solution, which had been uncultured, that is the eye-spot fungus had not been grown upon it and it had been kept sterile. In these solutions were placed leaves of similar age from a similar stalk of H 109 sugar cane. These bottles served as controls on the eye-spot filtrate.

In order to compare the reaction of the filtrate on leaves of a cane variety resistant to eye spot as compared to leaves of the susceptible H 109, leaves were cut from a fresh stalk of the resistant Yellow Caledonia variety, in consecutive ages as with the H 109, and placed in the same bottles as the H 109 leaves. The experiment was set up at 4:00 P. M., June 2nd.

On June 3rd, at 4:00 P. M., there was no wilting of the leaves in either the filtrate or control bottles, but there was a decided reddening of the vascular bundles of the leaves in the filtrate not visible in the leaves of the controls. This reddening seemed to be more noticeable in the Yellow Caledonia leaves than in the H 109 leaves.

On June 4th, at noon, there was a decided wilting of the H 109 leaves in the filtrate which was not evident in the Yellow Caledonia leaves in the filtrate or in either the Yellow Caledonia or H 109 leaves in the controls. The results at this time were recorded photographically. At the time of taking the photograph the leaves were removed from the filtrate and culture solution controls and placed in water.

On June 5th, at 7:00 A. M., there was wilting of both the Yellow Caledonia and H 109 leaves previously in the filtrate but no wilting of either the Yellow Caledonia or H 109 leaves previously in the culture solution controls.

On June 6th, at 9:00 A. M., the results were the same except that the leaves of both H 109 and Yellow Caledonia in the culture solution controls had begun to wilt.

Tentative conclusions.—(1) It seems evident that there is a toxic substance produced by the eye-spot fungus which if grown in liquid culture media will diffuse out into the culture solution and can then be separated from the fungus by filtration through a Chamberland filter.

(2) Yellow Caledonia leaves reacted to the toxic substance considerably more slowly than the H 109 leaves but eventually did react.

Experiment VI

The effect of heat upon the toxic substance of the eye-spot fungus.—The filtrate of the culture of the eye-spot fungus in Richard's solution which was used in the experiment of June 2nd, was used in this test. This was done since it was a difficult procedure to separate the toxic substance

from the fungus and it was thought that the filtrate in the previous experiment could be used again.

This filtrate was therefore sterilized in the autoclave at fifteen pounds pressure for twenty minutes. Such an exposure to heat would almost certainly destroy enzyme action and would probably coagulate most proteins. The presence or absence of a reaction to this autoclaved filtrate would therefore indicate whether the toxic substance was of a protein or non-protein nature.

In order to determine more fully the effect of the toxic substance on H 109 leaves of different ages, the youngest leaf, second, third, sixth and ninth leaves on a single stalk were cut off and their bases immersed in the toxic solution. Leaves in the same order of age were also selected from another stalk and placed with their butts in a non-cultured Richard's solution as controls.

June 8th, this experiment was set up at 11:50 A. M.

June 9th, there was no obvious effect at 7:00 A. M. At 3:00 P. M. youngest leaf in filtrate showed wilting; no wilting in other leaves.

June 10th, 7:00 A. M., all leaves were definitely wilted in filtrate, but all leaves remained normal in controls. Wilted leaves also showed reddening of vascular bundles nearest to the midribs.

Tentative conclusions.—(1) It seemed evident that the toxic substance was not of an enzyme nature nor of a protein nature for the exposure of the toxic substance to the autoclave would have destroyed enzymes and coagulated most proteins.

(2) The results rather indicated the presence of a simple inorganic toxic substance as a by-product of the activities of the eye-spot fungus.

Experiment VII

To corroborate the previous experiment showing the non-protein nature of the eye-spot toxic substance.—The filtrate of the 50-day old culture of the eye-spot fungus in Richard's solution was autoclaved again for 20 minutes at 15 pounds pressure and then diluted with equal parts of distilled water.

June 17th: The experiment was set up at 6:00 P. M. with leaves on stalk in order of their age; 1st, 2nd, 3rd, 6th and 9th. Leaves of the same age were placed in diluted uncultured medium of identical character as controls.

June 18th, noon: Slight wilting of leaves occurred in the filtrates; little or no discoloration was evident.

June 19th, 9:00 A. M.: The oldest (9th) leaf in the filtrate was almost entirely yellow; 6th leaf yellowed but not as much as 9th; also wilted.

1st, 2nd and 3rd leaves in age were all wilted and rolling at edges, but there was no yellowing as yet.

All controls were normal.

June 20th, 7:30 A. M.: All leaves in the filtrate were badly wilted; leaves in controls entirely normal; the results are shown in fig. 1.

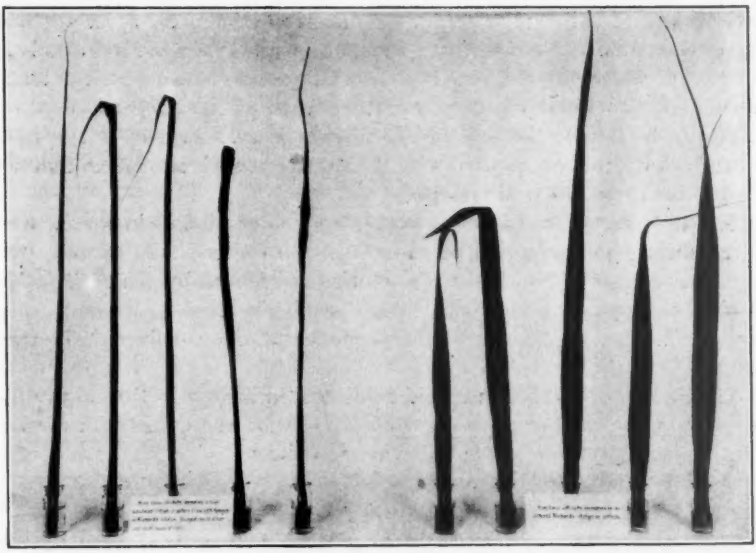


FIG. 1. Effect upon the leaves of the H 109 cane variety of the toxic substance in filtrates of cultures of the eye-spot fungus in Richard's solution. Left: H 109 leaves with butts immersed in twice autoclaved filtrate of culture of eye-spot fungus in Richard's solution. Youngest leaves at left and oldest leaves at right. Right: H 109 leaves with butts immersed in uncultured Richard's solution as controls. The photograph shows the toxic substance still active after autoclaving.

June 23rd, 8:00 A. M.: Experiment concluded.

Conclusion.—The toxic substance of the eye-spot fungus is apparently of a non-protein nature since after being subjected to a high heat its effect upon H 109 leaves is not at all minimized.

EXPERIMENT VIII

TO DETERMINE THE PRESENCE OF NITRITES IN CULTURE FILTRATES.—As a student the present writer had been associated with an investigator (PIERRE BONCQUET) who, in investigating several different plant diseases, showed the presence of considerable concentrations of nitrites within the lesions of

such diseases. The correlation of the presence of nitrites with such other plant diseases also suggested itself as of possible interest in the case of eye spot of sugar cane. Using the tests described in the Manual of the American Society of Bacteriologists,[2] determinations for the presence of nitrites were therefore made in the filtrates from eye-spot cultures, and as controls, tests were made on the uncultured Richard's solution. The tests were as follows: 5 gm. of sulphanilic acid were dissolved in 150 cc. of acetic acid (sp. gr. 1.04) to make solution A. 1 gm. of α-naphthyl-amine was dissolved in 22 cc. of water, filtered, and 180 cc. of dilute acetic acid added to make solution B. To test for nitrites add 2 cc. each of A and B to 10 cc. of culture to be tested; the presence of nitrites is evidenced by a rose-pink color which should be compared with the absence of such color from controls when tested in an identical manner.

The tests showed considerable concentrations of nitrites in the filtrates of the culture solutions and no nitrites in the controls. Apparently the potassium nitrate of the Richard's solution is reduced by the *Helminthosporium* of sugar-cane eye-spot disease, forming nitrites easily and abundantly. The test was repeated several times and on a number of different cultures.[3]

The finding of nitrites in considerable concentrations in the culture filtrates and the hitherto rather uncertain association of nitrites with certain plant diseases gave rise to the following experiment.

Experiment IX

To test the toxicity of dilute solutions of sodium nitrite versus sodium nitrate on H 109 leaves.—The filtrates of cultures of the eye-spot fungus in Richard's solution having been shown to contain a toxic substance of a non-protein nature, and having shown a marked reaction when tested for nitrites, the present test was inaugurated to show if simple nitrites would show the eye-spot reaction as much as the toxic substance from culture filtrates. Sodium nitrate solution of the same concentration as the sodium nitrite solution was used as a check.

June 13th, 4:30 P. M.: Five bottles containing 0.5 per cent. of sodium nitrite in distilled water were used in which to immerse the butts of H 109 leaves. Five bottles of sodium nitrate of the same concentration in distilled

[2] This manual has not been available to the writer in Manila so that the page reference cannot be given. However, the same test is given in Muir, Robert, and Ritchie, James. Manual of Bacteriology, p. 356. Oxford University Press, 1921.

[3] At this time Mr. Arthur F. Bell, of the Bureau of Sugar Experiment Stations of the Queensland Government, was a visitor in the laboratory and appreciation is here expressed to Mr. Bell for the considerable assistance rendered by him in making these tests.

water were run with H 109 leaves as controls. The leaves were placed in these solutions as follows: 1, 2, 3, 6 and 9, in order of their age on the stalk; 1 was the youngest leaf.

June 14th, 7:30 A. M.: The leaves in the sodium nitrite solution were slightly wilted and did not have the luster of leaves in the nitrate solution.

June 14th, 2:00 P. M.: The leaves in nitrite solution showed definite reddening along the midribs.

June 14th, 4:00 P. M.: The wilt was considerably advanced and vascular bundles alongside midribs were definitely reddened in the nitrite solution; leaves in the nitrate solution were entirely normal. The younger leaves were wilted more than older leaves but reddening was equally pronounced in all leaves.

June 15th, 8:00 A. M.: The reaction of the cane in the sodium nitrite was more pronounced; reddening of such leaves was also more pronounced and very definite.

June 16th: Experimental results photographed and shown in fig. 2.

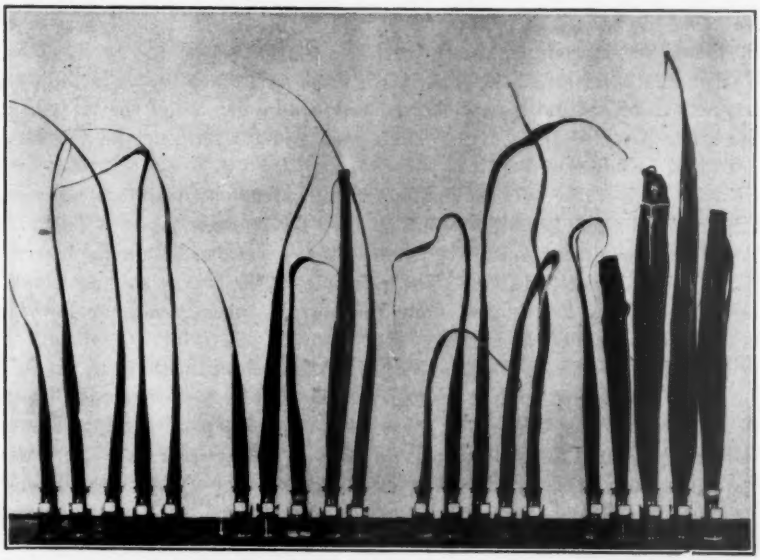


FIG. 2. The effect of the filtrate of the eye-spot fungus in Richard's solution on leaves of H 109 and Yellow Caledonia. In each series there were five leaves in order of their age on the cane stalk; youngest to the left, oldest to the right. The series of five leaves to the left consisted of H 109 leaves in the filtrate; the series to the right were H 109 controls; the third series were Caledonia leaves in the filtrate and the fourth series at the right were Caledonia controls. Photographed four days after being placed in filtrate.

Conclusions.—Solutions of sodium nitrite produced a reaction in H 109 leaves, closely similar to the reaction of the toxic filtrate of cultures of the eye-spot fungus in Richard's solution.

Experiment X

To substantiate results obtained previously with sodium nitrite solutions which gave a typical eye-spot reaction on H 109 leaves, with reduction of chlorophyll, while leaves in sodium nitrate solution as controls remained normal.—June 23rd, 9:00 A. M.: Three solutions were prepared, the first containing 0.125 per cent. of sodium nitrite in water, the second containing 0.25 per cent. of sodium nitrite in water and the third, 0.25 per cent. of sodium nitrate in water.

Three stalks of H 109 were cut and on each stalk the first, second, third, sixth and ninth leaves in order of their age in the stalk were cut, first being the youngest and ninth the oldest.

Immediately after cutting off these leaves, their butts were immersed in the solutions, the leaves of one stalk in 0.125 per cent. sodium nitrite, the leaves of the second in 0.25 per cent. sodium nitrite and the leaves of the third in 0.25 per cent. sodium nitrate.

June 24th, 8:00 A. M.: The third, sixth and ninth leaves in both concentrations of sodium nitrite showed reddened color along the midribs, in the lower parts of the leaves. There was a slight tendency of the leaves to roll at the edges and wilt.

June 25th, 7:30 A. M.: The older leaves were obviously more severely affected by the sodium nitrite solutions than the younger leaves. The leaves in the 0.25 per cent. solution were more severely affected than the leaves in the 0.125 per cent. solution. The reaction of the leaves was as usual, a reddening of the leaf blades along the midribs, usually more noteworthy in the lower parts of the leaves.

June 26th, 9:30 A. M.: The second, sixth and ninth leaves in the 0.125 per cent. solution of sodium nitrite showed definite yellow streaks typical of the earlier stages of eye spot; these yellow streaks were not found in the more concentrated, 0.25 per cent., solution of sodium nitrite. There were present in both concentrations the red streaks typical of the more advanced stages of eye spot. The control leaves in sodium nitrate solutions were entirely normal.

June 27th: The leaves were now so wilted and rolled as to obscure the yellow and red streaks of the leaves in the sodium nitrite solutions, and the experiment was discontinued.

Conclusions.—Sodium nitrite solutions reproduced the yellow and red streaks in H 109 leaves typical of eye spot. The reaction of sodium nitrite on the leaves was identical with the reaction of the filtrate of cultures of the

eye-spot fungus in Richard's solution. Since Richard's solution contains nitrates which when cultured with the eye-spot fungus produce abundant nitrites, there is a strong inference that the action of the eye-spot fungus on H 109 leaves is to reduce nitrates to nitrites which in turn react on the leaves to form the typical yellow and red streaks which result from eye-spot infection.

The action of the eye-spot fungus and of the sodium nitrite solutions is clearly a reduction of the chlorophyll to form first the yellow color and, with greater concentration, the red color observed in the leaves.

Experiment XI

To test the reaction of thrice-autoclaved, diluted filtrate of the eye-spot fungus in Richard's solution on H 109 leaves.—June 27th: Using the same filtrate of cultures of the eye-spot fungus in Richard's solution, having been autoclaved once more however, making three times in all, an experiment was set up to test the effect of dilution on the filtrate.

One part of the filtrate was diluted with equal parts of water, making a 50 per cent. concentration. The other part of the filtrate was diluted with 3 parts of water, making a 25 per cent. concentration. As a control, uncultured Richard's solution was diluted to a 50 per cent. concentration with water.

June 27th, 4:30 P. M.: Five H 109 leaves were placed in the 25 per cent. concentration of the filtrate; leaves 1, 2, 3, 6 and 8 in order of their age on the stalk. Five leaves from another stalk in order of their age 1, 2, 3, 5 and 8 were placed in a 50 per cent. concentration of the filtrate. Five leaves from another stalk, leaves 1, 2, 3, 5 and 8 in order of their age, were placed in a 50 per cent. concentration of uncultured Richard's solution as controls.

June 28th, 7:00 A. M.: No results were evident as yet.

June 29th, 7:00 A. M.: In the 50 per cent. concentration of filtrate, leaves 3, 5 and 8 were rolled and slightly wilted but not discolored, while leaves 1 and 2 were still normal. In the 25 per cent. concentration of filtrate, leaves 6 and 9 were slightly wilted and edges rolled; leaf 9 was decidedly yellow, especially at the tip; leaf 6 also was yellow at the tip, but less so than 9; other leaves were normal. Control leaves were normal.

June 30th, 7:30 A. M.: All leaves in both 25 and 50 per cent. concentrations of the filtrate were severely wilted; control leaves were normal.

July 1st: The leaves the same as on June 30th; the experiment discontinued.

Conclusions.—The results of this experiment showing that the filtrate of the cultures was still toxic after three exposures to high heat in the autoclave indicated that the toxic substance was of a non-protein nature. The

results also indicated that older leaves of H 109 were more susceptible to the toxic substance than young leaves. Finally, the experimental results also indicated that a more typical eye-spot reaction was obtained with a diluted toxic substance than with the more concentrated form.

Experiment XII

To substantiate a previous experiment showing that dilute sodium nitrite solutions produce a more typical eye-spot reaction on H 109 leaves than stronger concentration.—July 1st: Solutions were prepared containing 0.125 and 0.25 per cent. of sodium nitrite in water and 0.25 per cent. of sodium nitrate in water.

5:30 P. M.: From a fresh stalk of H 109, counting the youngest, newest unfolded flat leaf as leaf number 1, were cut off leaves numbers, 2, 3, 6, 7 and 9 and their butts immediately immersed in the solution of 0.25 per cent. sodium nitrate. From a similar stalk of H 109 a series of leaves of identical ages were cut and placed in the solution of 0.25 per cent. sodium nitrite. A similar series of leaves of identical ages was cut and the leaf butts immediately immersed in solutions of 0.125 per cent. of sodium nitrite.

July 2nd, 8:00 A. M.: No reaction in any series.

July 3rd, 8:00 A. M.: In the 0.25 per cent. solution of sodium nitrate there was no reaction. In the 0.25 per cent. solution of sodium nitrite all leaves were slightly rolled and showed red streaks, older leaves were more severely affected than the younger leaves. In the 0.125 per cent. solution of sodium nitrite the same reaction was recorded but the reddening was confined to the leaf blades near the midrib at the bases of the leaves.

July 4th, 6:00 P. M.: Fine examples of typical yellow eye-spot streaks were observed in leaves, 2, 3, 7 and 8 in the 0.125 per cent. sodium nitrite solution and in leaves 3, 6 and 7 of the 0.25 per cent. solution of sodium nitrite. The controls in the sodium nitrate solution showed no reaction.

Conclusions.—Sodium nitrite solutions reproduced the yellow and red streaks in H 109 leaves typical of eye spot. Previous experiments and conclusions were corroborated. The older leaves appeared to be more susceptible to the toxic substance than the younger leaves.

Experiment XIII

To compare various nitrogen compounds as sources for the toxic substance.—In the earlier stages of this experimental work the possibility had suggested itself that since the toxic substance did not react to any appreciable extent in filtrates of cultures of the eye-spot fungus in beef bouillon, while there was an appreciable reaction in the filtrates of cultures in Richard's solution, possibly there was a buffering action of the proteins of the beef-extract bouillon against the toxic substance. This did not seem so

likely after the finding that the toxic substance was principally an inorganic nitrite, however the following experiment was inaugurated before this latter finding was established; the experiment was planned to test the formation or reaction of the toxic substance in filtrates of cultures with various sources of nitrogen and to test the buffering effect of beef-extract proteins on the toxic substance.

Richard's solution was prepared, omitting the potassium nitrate, and divided into five different lots. To one lot was added one per cent. of potassium nitrate; to the second lot one per cent. of asparagin; to the third lot one per cent. of peptone; to the fourth lot one per cent. of beef extract, and to the fifth lot one per cent. of potassium nitrate plus one per cent. of beef extract. There were therefore four different sources of nitrogen of increasing complexity of structure, and in the fifth lot there was a mixture of protein nitrogen with inorganic nitrogen to determine whether or not such protein would have a buffering action upon the toxic substance. All five classes of media were then sterilized in the Arnold sterilizer on three successive days for 20 minutes of steaming each day.

There were 12 Erlenmeyer flasks of each medium and 6 flasks of each medium were inoculated with the eye-spot fungus, the remaining 6 flasks of each series being kept as controls; this experiment was set up on May 19th, 1927.

On August 20th, 92 days later, all cultures were filtered through Chamberland filters, each series of a distinct medium having a separate clean Chamberland filter; the filtrate was received in sterile flasks.

The filtrates of all series of the cultured media were then tested separately for the presence of nitrites, using the sulphanilic acid, α-naphthylamine test; the results were as shown in table I.

TABLE I

PRESENCE OR ABSENCE OF NITRITES IN FILTRATES OF EYE-SPOT CULTURES, AND UNCULTURED CONTROLS, IN MEDIA WITH VARIOUS SOURCES OF NITROGEN

	POTASSIUM NITRATE	ASPARAGIN	PEPTONE	BEEF EXTRACT	POTASSIUM NITRATE PLUS BEEF EXTRACT
Filtrates of cultures	++	Trace	–	–	+
Uncultured media as controls	–	–	–	–	–
Weight of mycelial mass filtered from culture: grams	4.24	5.01	6.19	4.72	5.57

The tests showed a very strong presence of nitrites in the medium with potassium nitrate as a source of nitrogen; a lesser reaction for nitrites in

the medium with mixed potassium nitrate and beef bouillon and but a trace of reaction from the medium containing asparagin. There was no reaction for nitrites in the media containing peptone or beef extract alone.

In this connection it is interesting to note that the mycelial masses from the different series of media were weighed and for convenience such weights are also given in table I. These figures show that there is no correlation between quantity of growth and formation of the toxic nitrites. Rather it is evidently the source of nitrogen which is the factor governing the formation of nitrites. Apparently the fungus of eye spot of sugar cane grows more vigorously on organic forms of nitrogen than on inorganic forms. As against this, however, the fungus seems to have little effect in reducing such more complex forms of nitrogen to nitrites but does have a strong capacity to reduce inorganic nitrates to nitrites; apparently it is this latter factor in its physiology which causes it to be a serious pathogen to certain of the sugar-cane varieties.

The filtrates of the different series of culture media were then diluted with equal parts of sterile distilled water, placed in the straight-sided bottles used in previous experiments, and five leaves of similar degrees of age from identical stalks of the variety H 109, placed with their cut ends or butts in such filtrates. The results of this test are shown in table II.

The results of these toxic substance reaction tests correlate closely with the results of the tests for nitrites in these filtrates and controls, the one exception being that, whereas the qualitative determination for nitrites by the sulphanilic acid test gave no reaction in the filtrates of cultures in peptone medium, there was a slight reaction on the part of the leaves in such filtrates, indicating the presence of this toxic substance; apparently, then, as deduced from previous experiments only very slight quantities of nitrites are necessary to produce a reaction on the leaves of a susceptible cane variety, and one would judge that the leaves give a more delicate test for the presence of nitrites than the sulphanilic acid method.

Conclusions.—The results tabulated in table II would indicate that the eye-spot fungus can produce the toxic substance for H 109 leaves in a medium containing nitrates; this toxic substance is not materially buffered in the presence of the proteins of beef bouillon as was at one time in these studies thought possible; the toxic substance is produced to a lesser extent in a medium containing asparagin, and to an even lesser extent in a medium containing peptone; the toxic substance was not produced in a medium containing beef extract as the sole source of nitrogen.

Review of literature and discussion

The literature concerning the toxic substances formed by plant pathogens is fairly extensive, and no effort will be made to present a complete

TABLE II

REACTION OF LEAVES OF SUGAR CANE, H 109 VARIETY, TO FILTRATES OF 92-DAY-OLD EYE-SPOT CULTURES IN VARIOUS MEDIA. EXPERIMENT SET UP AT 9:30 A. M., AUGUST 27TH, 1927

DATE	POTASSIUM NITRATE UNCULTURED CONTROLS	FILTRATE OF POTASSIUM NITRATE CULTURES	FILTRATE OF ASPARAGIN CULTURES	FILTRATE OF PEPTONE CULTURES	FILTRATE OF BEEF EXTRACT CULTURES	FILTRATE OF POTASSIUM NITRATE PLUS BEEF EXTRACT CULTURES
Aug. 28th, 8:00 A. M.	All normal	All normal	All normal	All normal	All normal	All wilted[2]
5:00 P. M.	All normal	All wilted	All wilted	All slightly wilted	All normal	All normal
Aug. 29th, 7:00 A. M.	All normal	All wilted[1]	All wilted not yellow	All wilted[2]	All normal	All wilted

[1] Leaf 8 shows yellow streaks typical of natural eye-spot lesions; all other leaves are wilted without yellowing.

[2] Leaves 6 and 8 completely yellowed as in natural eye-spot lesions.

bibliography of the subject, but mention of a few recent papers will suggest the most recent viewpoints.

BRANDES (1) working on the *Fusarium* wilt of banana plants in 1919 expressed what seemed to be the viewpoint from gradually accumulating evidence at that time for several wilt diseases, that "wilting is not due to plugging of the vessels by mycelium, but is probably the result of toxic excretions by the fungus."

WHITE (7) showed the presence of toxic substances in filtrates of cultures of *Fusarium lycopersici*. He apparently arrived at the conclusion that there were two toxic principles in such filtrates; the first, of the nature of an enzyme, and the second crystalloidal and thermostable. He believed that among the organic acids present in the filtrates, might be found the thermostable toxic substance.

ROSEN (5) showed that sterile filtrates of cultures of *Fusarium vasinfectum*, the pathogen of cotton wilt, in Richard's solution (nitrogen in the form of nitrates) were toxic to cotton plants. Filtrates of cultures of the same organism in Uschinsky's solution (nitrogen in the form of asparagin) or peptone bouillon (nitrogen in the form of peptones) were non-toxic to cotton plants. Whole plants with normal uninjured roots when placed in filtrates of cultures in Richard's solution, exhibited the results of the toxic substance, showing absorption of the toxic substance through the roots. These toxic filtrates gave positive tests for nitrites and using chemically pure sodium nitrite solutions of concentrations comparable to the concentrations found in the culture filtrates, ROSEN obtained a reaction by his cotton plants similar to the toxic effect exhibited by his culture filtrates. ROSEN also came to the conclusion that there were two toxic substances formed by the *Fusarium* of cotton wilt: the first was an inorganic salt in the form of a nitrite and the second a volatile compound with an alkaline reaction.

In his paper, ROSEN suggests that cotton wilt which is more prevalent in his experience on sandy soils may be correlated with the lack of organic nitrogen in such soils, basing this deduction on the presence of the toxic substance in cultures containing inorganic nitrogen and the absence of the toxic substance in cultures containing only organic nitrogen. He goes somewhat further along this line of thought in a later note (6) in which he suggests the possibility of the control of cotton wilt by the use of organic nitrogen fertilizers. The work of BRANDES, WHITE and ROSEN, all showed that the wilting produced in plants placed in culture filtrates as compared to normal plants in controls, could not have been brought about by a change in osmotic pressure of the culture solution which might have resulted from the fungus metabolism.

The results of studies outlined in the present paper are in close agreement with the main body of the conclusions by WHITE and by ROSEN

although there is evidence of the presence of but one toxic substance from the studies here recorded. The toxic substance produced by the fungus of eye-spot of sugar cane has been shown to be a soluble, inorganic substance not affected by heat, and subsequently by identical reactions the inference is strong that this substance is simply an inorganic nitrite. These nitrites are formed readily in media containing inorganic nitrates and to a less extent from lower organic nitrogen forms, such as asparagin, but are not formed at all from peptone, or the higher protein forms of nitrogen. The toxic action of the nitrites is not buffered by the presence of proteins such as those in beef extract.

Apparently the toxic substance is not truly a metabolism product of the pathogen but results solely from the vigorous capacity of the pathogen to reduce to nitrites the inorganic nitrates present in the medium or other environment.

These results obtained in culture and toxin studies fit in admirably with the results by LEE and MARTIN (3) which showed big increases in the virulence of the disease following applications of inorganic-nitrogen fertilizers to fields of susceptible cane varieties, even though the nitrogen is applied in the form of ammonium sulphate rather than sodium or potassium nitrates. Apparently the nitrogen taken up by the cane plant arrives in the cane foliage in the form of nitrates, which are reacted upon vigorously by the eye-spot fungus to produce nitrites abundantly, which are in turn toxic to the cane-leaf tissues. One would judge that one important effect of the nitrates on the leaf tissues was the reduction of the chlorophyll as indicated by the color changes from green to yellow and red in streaks on the leaves. It is of possible physiological interest that this reduction takes place more strongly in the older leaves than in the younger ones.

As to the application of nitrogen in organic forms to minimize the disease or more properly to avoid the formation of toxic substances as suggested by ROSEN, the question would reasonably seem to be, whether such nitrogen arrives in the cane leaves in the form of organic compounds or as inorganic nitrates. The field experience with ammonium sulphate and with organic forms of nitrogen fertilizers, such as filter-press mud from sugar-cane factories, or dried blood, etc., has shown the big increases of eye-spot similar to the increases of the disease when inorganic nitrate fertilizers are used, and would seem in opposition to the theory that organic fertilizers minimize the disease.

There is, however, a field application of these experimental results, which has already proven notably successful; when the application of nitrogen fertilizers is made at such a time as to bring the physiological response in the host plant, at a time of the season when climatic conditions for fungus

infection are at a minimum there is scarcely any injury from the disease at all. This has been proven out as a commercially feasible practice in the case of eye-spot of sugar cane.

The tests with the toxic filtrates on leaves of the Yellow Caledonia variety, a variety which is resistant to eye-spot, also led to some interesting inferences. Yellow Caledonia leaves frequently become infected under natural field conditions, but never develop the long streaks of killed tissue found on the susceptible varieties following infection; instead, there are formed only small local lesions at the point of infection. However, when Yellow Caledonia leaves are placed in solutions of the toxic substance, they indubitably react to such a toxic substance. One would conclude therefore that studies of the nitrogen metabolism of a variety such as Yellow Caledonia would possibly show the nitrogen in the leaves, in forms other than the simple inorganic nitrates, so that the fungus had difficulty in producing the toxic nitrites.

At the time of the conclusion of the writer's work on eye-spot, attention was also called to the possibility of fertilizing with catalytic oxidizing agents in an effort to get such catalysts into the nutrient streams of the plant in order to neutralize the reducing effect upon nitrates produced by the pathogen. The salts of such metals as iron, manganese and zinc were suggested for such attempts.

The studies at least lead to a more intelligent attempt at producing in the host plant resistance to the toxic effects produced by a certain group of plant pathogens.

Summary

1. Tests for a toxic reaction of susceptible sugar-cane leaves were conclusively negative when tested with filtrates of cultures of the fungus of eye-spot of sugar cane, *Helminthosporium sacchari*, in pure sucrose solutions and in beef-extract bouillon media.

2. Tests on sugar-cane leaves with filtrates of cultures of the same fungus in Richard's solution showed the presence of a toxic substance. This toxic substance was shown to persist after heating to an extent which would destroy enzymes and coagulate most proteins. Attention rather naturally therefore turned towards simpler nitrogen compounds as the toxic substances.

3. The presence of considerable concentrations of nitrites was shown in the filtrates of cultures of the fungus in Richard's solution and to a lesser extent in an asparagin medium, but such nitrites were not evidenced in filtrates of cultures in a peptone bouillon medium nor in a beef-extract bouillon, nor in uncultured Richard's solution as a control on the test for nitrites.

4. Susceptible sugar-cane leaves evidenced a toxic reaction from filtrates of the cultures in Richard's solution, to a lesser extent in an asparagin medium and to a much slighter extent in a peptone medium. There was no toxic reaction by such leaves to filtrates of cultures in beef-extract bouillon. There was no correlation between quantity of growth in these media, and quantity of the toxic substance produced. The reaction of cane leaves to these filtrates of cultures in media with various nitrogen sources was closely correlated with the presence of nitrites in such filtrates.

5. Dilute solutions of potassium nitrite brought about the same reaction of susceptible sugar-cane leaves as the filtrates of cultures of the eye-spot fungus in media containing the lower nitrogen compounds. This reaction, in dilute concentrations, was a yellowing of leaves either totally or in streaks, and sometimes the formation of brownish-red streaks up and down the leaves. Leaves in these filtrates always wilted and showed a loss of the glossy appearance typical of the control leaves which remained unwilted and a normal green color.

6. The evidence points to this toxic substance, therefore, not as a direct product of the metabolism of the fungus but as a product of the environmental medium. Apparently the fungus of eye-spot of sugar cane has a strong capacity to reduce to nitrites some of the lower nitrogen compounds in its environmental medium; these nitrites in turn are toxic to the leaf tissues of the host plant. One notable toxic effect produced by such nitrites is the reduction of the chlorophyll of the host tissues.

7. The results recorded in this paper indicate a strong relationship between the virulence of this disease, and probably many other plant diseases, to the nitrogen nutrition of the host plant. The attempt to apply these results to commercial crop production by using organic nitrogen fertilizers for the nutrition of the host plants depends for its success on the nitrogen from such fertilizers reaching the leaves in a nitrogen form other than as nitrates. Field results with organic nitrogen fertilizers in the case of eye-spot disease of sugar cane indicate as large an increase of the disease as that which follows applications of inorganic nitrogen fertilizers. The logic of the results recorded in this paper as well as of the field results does not therefore indicate that applications of organic nitrogen fertilizers will serve as a control measure for this disease.

8. A practical application has been developed for minimizing this disease which in principle is dependent on avoiding nitrogen fertilizers during seasonal periods in which climatic conditions favor fungus infection; quite the reverse, nitrogen fertilizers of any class should be applied at a time when the host plant will react to the nitrogen in a period with climatic

conditions unfavorable to infection. This procedure has been notably successful in actual practice against eye-spot disease of sugar cane.

PHILIPPINE SUGAR ASSOCIATION,
MANILA, PHILIPPINE ISLANDS.

LITERATURE CITED

1. BRANDES, E. W. Banana wilt. Phytopath. **9**: 339–389. 1919.
2. LARSEN, L. D. The eye-spot disease. Hawaiian Planters' Record **7**: 18–30. 1912.
3. LEE, H. ATHERTON, and MARTIN, J. P. Effect of fertilizer constituents on the eye-spot disease of sugar cane. Jour. Ind. Eng. Chem. **20**: 220–224. 1928.
4. MARTIN, J. P., and LEE, H. ATHERTON. The time of application of nitrogen fertilizers in relation to eye-spot disease. Hawaiian Planters' Record. **32**: 9–11. 1928.
5. ROSEN, H. R. Efforts to determine the means by which the cotton-wilt fungus, *Fusarium vasinfectum*, induces wilting. Jour. Agr. Res. **33**: 1143–1147. 1926.
6. ————. The control of cotton wilt by the use of organic fertilizers. Science n. s. **65**: 616–617. 1922.
7. WHITE, RICHARD P. Studies on tomato wilt caused by *Fusarium lycopersici* Sacc. Jour. Agr. Res. **34**: 197–239. 1927.

THE ABSORPTION OF PHOSPHATE FROM SOIL AND SOLUTION CULTURES[1]

L. J. H. TEAKLE

(WITH FOUR FIGURES)

Introductory

In the study of the phosphate nutrition of plants it is of importance to know what relation exists between the soil solution and the absorption of phosphate by the growing plant, concerning the power to do which, there is a difference of opinion. Some emphasize the effect of carbon dioxide arising from the respiration of the roots; others suggest an internal factor related to the metabolism of the plant. The question of the rate of supply as well as the effect of concentration is of great importance in the interpretation of results of soil and solution culture experimentation. This has been overlooked by certain of the earlier plant physiologists and has led to an emphasis of the importance of the action of the roots on the soil particles. There is, however, ample experimental evidence to show the ability of plants to absorb phosphate from solutions comparable in phosphate concentration with the soil solution. Thus BIRNER and LUCANUS (**4**) and BEYER (**3**) as early as 1870 had shown the ability of oat plants to make normal growth in well water containing 2 ppm. PO_4 and SCHLOESING fils (**13**) and KOSSOWITCH (**9**) arrived at similar conclusions some thirty years later. SCHLOESING fils found 1 ppm. PO_4 adequate for certain plants and concluded that the soil solution supplied at least the major part of the phosphate absorbed from the soil. KOSSOWITCH recognized a difference between flax and mustard and suggested that this was due to effect of the carbon dioxide formed by the roots. HOAGLAND and MARTIN (**7**), using solution cultures, obtained good growth of barley at 0.7 ppm. PO_4. Somewhat better growth occurred in similar solutions containing 2.4 ppm. and 9.0 ppm. PO_4.

The rôle of calcium carbonate in limiting absorption from rock phosphates has been indicated by PRIANISCHNIKOW (**12**), BREAZEALE (**5**) and others. Apparently the carbonic acid arising from root respiration is neutralized by the calcium carbonate with the formation of calcium bicarbonate which depresses the solubility of calcium phosphate. It seems that carbonic acid *may* be an important agent in the solution of soil phosphates, but

[1] Contribution from the Laboratory of Plant Nutrition, the University of California. This problem was suggested by Professor JOHN S. BURD, to whom grateful acknowledgment is made for helpful counsel throughout the investigation.

in highly buffered, neutral or slightly alkaline soils the plant must draw its supply from the phosphate of the soil solution. A recognition of this difference may lead to an explanation of the different results obtained under varied circumstances and to a reconciliation of various theories.

Experimental

This series of experiments was designed to afford evidence concerning the relation between the phosphate concentration of the soil solution and the absorption of that ion by the wheat plant.

For this purpose, Pusa 4 wheat, obtained from the University Farm, Davis, California, was used on account of its early maturity. It was grown in soil cultures and in solution cultures, and, after harvesting, the plants were weighed and absorption determined by analysis. All experiments here reported were conducted during the summer of 1926 in the same greenhouse at the University of California, Berkeley, California. The methods in common use in the laboratories of Plant Nutrition, University of California, were employed throughout. Brief mention may be made as follows:

The soil solution was obtained by the water displacement method of BURD and MARTIN (**6**).

Phosphate, when in sufficient concentration, was determined by titration of the phospho-molybdate precipitate. For small concentrations, and for the control of the solution cultures, the coeruleo-molybdate method described by ATKINS (**2**) was used.

To obtain the ash for analysis, samples of the dried and ground plant materials were weighed, mixed intimately with magnesium oxide, and ignited to a dull red heat in an electric muffle.

A. Soil cultures

Three soils were selected for the cultural experiments on account of their physical condition and the phosphate concentration of the soil solution. They were all amenable to the displacement method. Certain details of their history and composition are given in table I.

It should be added that each soil was neutral or nearly neutral in reaction and exhibited a strong buffer action toward acid. Further details of this behavior are reported in another paper (**15**).

The soils were subjected to various treatments in order to modify the concentration of the soil solution with respect to phosphate. In the case of soils 29 and 30, the calculated amounts of hydrochloric acid and chlorides of calcium and magnesium were added to the distilled water used in moistening the dry soil.

On account of the poverty of soil 33 (see table II) a culture solution, 0.01 molal with respect to potassium, calcium, magnesium, and sulphate

TABLE I

DESCRIPTION OF SOILS USED THROUGHOUT THE EXPERIMENTATION

LABORATORY SERIAL	SOIL SERIES (CALIF. SURVEY)	ORIGIN	PAST TREATMENT	TOTAL PO_4 (DRY BASIS)
				Per cent.
29	Oakdale sandy loam	Stanislaus Co. J. Skittone Ranch, Salida, California	Uneropped edge of a vineyard	0.24
30	Fresno fine sandy loam	San Joaquin Valley, G. Christensen Ranch, Fresno, California	Originally grain Edge of vineyard during last 24 years	0.18
33	Not mapped, sandy loam	Creek bottom, University Farm, Davis, California	None	0.16
33a	Not mapped, sandy loam	Creek bottom, University Farm, Davis, California	Intimately mixed with 0.085 per cent. PO_4 as ground rock phosphate	0.24

TABLE II

SOIL 33. ANALYSIS OF THE DISPLACED SOLUTION

MOISTURE	SPECIFIC RESISTANCE	pH	PO_4	NO_3	Ca
Per cent.	*ohms*		*ppm.*	*ppm.*	*ppm.*
19	2250	7.8	0.2	52	50

and 0.03 molal with respect to nitrate, was used instead of distilled water to moisten the soil.

After standing for about one week, the cultures were seeded to obtain a stand of six healthy plants per culture. Three of these plants were harvested at the intermediate period. For each treatment, five two-gallon, glazed earthenware crocks, each containing 10 kilograms of soil, were used. The moisture was maintained by additions of distilled water in quantities determined by the loss in weight of the jars. Uncropped controls were used in connection with all experiments.

At the beginning of the experiment and at the two other periods during the growth of the plants, the soil solution was obtained from samples of soil under each treatment and from the controls. The results are shown in the accompanying tables and are considered to afford a fairly accurate picture of the fluctuations of the phosphate concentration of the soil solution under the several treatments.

The treatments of the soils and certain of the effects of these treatments on the composition of the soil solution are given in table III. All determinations were made in duplicate. The amendments were calculated on the basis of the theoretical soil solution and are expressed in terms of normality, or as parts per million of the soil solution. Another paper (TEAKLE **15**) deals more exhaustively with the chemical effects of these amendments.

In table IV are presented the data concerning the growth and analysis of the wheat plants under the several conditions. All ash analyses reported are the averages of closely agreeing triplicate determinations, and the weights given are averages of a harvest of at least 15 plants from each treatment.

Apparently healthy plants were produced, but there was a marked difference in tillering under the different treatments, which is reflected by the weights. No doubt the high salt content of the soil solution, induced by certain of the treatments, was a seriously complicating factor, and it will be impossible to use any of the data except those obtained under favorable conditions. It seems permissible to consider the effects of the treatments of soil 33 (including 33a), and also the results from soils 29 and 30 involving the milder treatments. Too little is known of the effect

TABLE III

SOILS 29, 30, 33. THE EFFECT OF AMENDMENTS ON THE COMPOSITION OF THE SOIL SOLUTION DISPLACED FROM SOILS WITH AND WITHOUT A CROP OF WHEAT, 1926

Soil	Chemical treatment	Crop	Planting				First Harvest				Final Harvest			
			Date	pH	PO_4	NO_3	Date	pH	PO_4	NO_3	Date	pH	PO_4	NO_3
					ppm.	*ppm.*			*ppm.*	*ppm.*			*ppm.*	*ppm.*
29	Distilled water	–	May 5	6.6	7.3	550	May 29	6.8	2.4	600	June 11	7.3	2.3	635
		+						7.2	2.6	21		7.8	4.4	12
29	0.08 N.HCl	–	May 5	6.2	12.4	218	May 29	6.1	8.6	265	June 11	6.9	5.0	525
		+						6.6	6.6	48		6.6	2.4	73
30	Distilled water	–	May 3	7.5	6.5	563	May 29	7.2	4.3	810	June 11	7.4	6.4	728
		+						7.8	4.5	55		8.3	3.8	17
30	0.03 N.HCl	–	May 3	6.8	12.8	551	May 29	6.8	9.6	785	June 11	7.2	11.0	680
		+						7.3	4.0	44		7.5	3.0	20
30	0.1 N.HCl	–	May 3	6.0	41.0	471	May 29	5.9	31.0	560	June 11	6.4	23.0	556
		+						6.4	19.0	350		6.8	13.0	180
30	1000 ppm. Ca as $CaCl_2$	–	May 8	7.1	3.6	362	May 29	7.2	3.0	790	June 16	7.5	4.1	888
		+						7.3	0.8	N.D.		7.3	1.6	22
30	500 ppm. Mg as $MgCl_2$	–	May 8	7.2	5.2	298	May 29	7.2	3.4	686	June 16	7.0	3.4	752
		+						7.0	0.9	N.D.		7.3	1.7	26
33	Culture solution minus PO_4	–	June 30	7.5	0.16	1760	.....	.....	.....	.....	August 12	7.4	0.19	1750
		+										7.6	0.15	276
33a	Culture solution minus PO_4	–	June 30	7.5	0.15	1870	.....	.....	.....	.....	August 12	7.5	0.17	1590
		+										7.8	0.21	126
33a	Culture solution plus 1390 ppm. PO_4	–	June 30	7.2	23.0	1800	.....	.....	.....	.....	August 12	7.4	11.1	1540
		+										7.8	12.5	108

TABLE IV

Soils 29, 30, and 33. Growth and composition of wheat plants in the soil under the treatments described in table III. Variety Pusa 4

Soil	Treatment	Date planted	First harvest						Final harvest					
			Date	Age	Soil solution PO_4	Plants			Date	Age	Soil solution PO_4	Plants		
						Dry weight	PO_4	PO_4 absorbed per plant				Dry weight	PO_4	PO_4 absorbed per plant
				days	*ppm.*	*gm.*	*per cent.*	*mg.*		*days*	*ppm.*	*gm.*	*per cent.*	*mg.*
29	Distilled water	May 5	May 29	24	2.6	0.36	3.10	1.12	June 11	37	4.4	1.01	2.76	2.79
29	0.08 N.HCl	May 5	May 29	24	6.6	0.14	4.45	0.62	June 11	37	2.4	0.87	3.40	2.96
30	Distilled water	May 3	May 29	26	4.5	0.48	2.75	1.32	June 11	39	3.8	1.84	1.96	3.61
30	0.03 N.HCl	May 3	May 29	26	4.0	0.48	2.75	1.32	June 11	39	3.0	2.07	1.92	3.97
30	0.1 N.HCl	May 3	May 29	26	19.0	0.22	4.40	0.97	June 11	39	13.0	1.03	3.27	3.37
30	1000 ppm. Ca as $CaCl_2$	May 8	May 29	21	0.8	0.24	2.54	0.61	June 16	39	1.6	1.88	1.64	3.08
30	500 ppm. Mg as $MgCl_2$	May 8	May 29	21	0.9	0.33	2.34	0.77	June 16	39	1.7	2.33	1.58	3.68
33	Culture solution minus PO_4	June 30	.....	.....	.....	.....	.....	.....	August 12	43	0.15	0.79	1.18	0.93
33a	Culture solution minus PO_4	June 30	.....	.....	.....	.....	.....	.....	August 12	43	0.21	0.90	1.18	1.06
33a	Culture solution plus 1390 ppm. PO_4	June 30	.....	.....	.....	.....	.....	.....	August 12	43	12.5	1.27	2.26	3.08

of balance of ions on absorption to permit of conclusions from results obtained under more severe conditions.

In considering soil 33 it seems apparent that the addition of a considerable amount of finely ground rock phosphate, which affected the concentration of the soil solution but little, had a correspondingly small effect on the absorption of phosphate by the plants. In soils of this nature the effect of carbon dioxide on the solid phase phosphate must be very small. In addition there appears to be no definite relation between the concentration of the soil solution and the amount of phosphate absorbed therefrom. The effect of concentration in this regard will be studied under more favorable conditions in solution culture, using concentrations suggested by a study of these and of other soils.

The treatment of soil 30 with $CaCl_2$ and $MgCl_2$ suggested that calcium in the soil solution retards the rate of solution of solid phase phosphate to replace that absorbed by plants. While the solubility of phosphate is lowered materially by the presence of increased amounts of calcium in the soil solution, according to the data in table V, it seems that this retardation is the more important effect in soil 30. It will be noted that on account of the phenomenon of base replacement the application of magnesium chloride gave practically the same results as an equivalent amount of calcium chloride.

B. Solution Cultures

It seemed desirable to conduct accurate controls in the shape of solution cultures. A constant flow device was therefore arranged as shown in fig. 1. Solution of carefully maintained composition was allowed to flow from the upper carboys (A) into the culture jars (B), being delivered at the bottom. The overflow was removed by means of a J siphon after the design of Anderssen (**1**) and caught in carboys (C) similar to the first. After analysis of the solutions in C, they were adjusted to the original concentrations and pumped into the upper carboys (A) by means of a small Nelson pump. The volume was adjusted with distilled water to 40 liters in these upper carboys. The rate of flow was controlled by glass stop cocks (D) and varied from 1 liter to 8 liters per day per plant depending on the composition of the solution and the age of the plants. It was found necessary to coat the containers and conducting tubes with a black asphaltum paint to exclude light. This necessitated a sheath of pasteboard or several thicknesses of brown paper to maintain the solutions at an even temperature. The culture jars (B) had a capacity of about 16 liters of solution and supported a growth of 10 or 20 wheat plants; the number being determined by the concentration of phosphate in the solution.

The carboys (A and C) each had a capacity of 40 liters and were graduated in units of two liters to facilitate control of the rate of flow. All

TABLE V

SOIL 30. THE EFFECT OF A HIGH CONCENTRATION OF CALCIUM IN RETARDING THE SOLUTION OF SOIL PHOSPHATE DURING THE GROWTH OF WHEAT PLANTS

SOILS MOISTENED MAY 6TH

	SOIL TREATMENT	ANALYSIS OF THE DISPLACED SOLUTION											
		MAY 8TH			MAY 29TH		JUNE 16TH, 1926						
		PO_4	Ca	Mg	PO_4		PO_4		Ca		Mg		
					CROP	UNCROPPED	CROP	UNCROPPED	CROP	UNCROPPED	CROP	UNCROPPED
		ppm.	*ppm.*	*ppm.*	*ppm.*	*ppm.*	*ppm.*	*ppm.*	*ppm.*	*ppm.*	*ppm.*	*ppm.*
1	Control	6.5	188	N.D.	4.5	4.3	3.8	6.4	84	240	30	67
2	1000 ppm. Ca as $CaCl_2$	3.6	1020	210	0.8	3.0	1.6	4.1	707	1080	141	209
3	500 ppm. Mg as $MgCl_2$	5.2	576	270	0.9	3.4	1.7	3.4	450	660	229	284

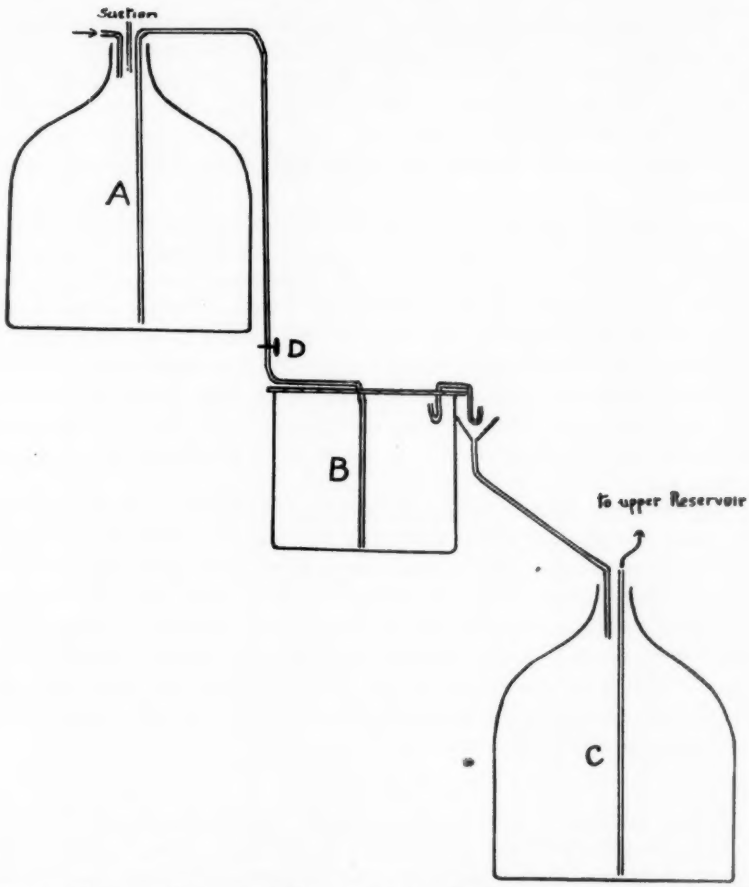


FIG. 1. Diagrammatic representation of a device for the constant renewal of culture solution used in the study of the growth of wheat.

experiments described were conducted side by side with the soil cultures described in the foregoing section.

Harvesting took place on the dates reported in table VI. The date of the final harvest was determined by the emergence of the anthers from the glumes; that is, the plants were harvested at the beginning of the flowering stage.

In all essentials the plants were treated in the same way as those grown in soil cultures.

Three experiments were conducted using this method and were designed to determine the growth and composition of wheat plants in solutions containing phosphate ranging from 0.05 ppm. PO_4 to 50 ppm. PO_4, the reaction being maintained at either pH 5.5 or pH 7.0. The results of these experiments are reported in table VI. The results of harvesting at an intermediate stage are not included in this table as not contributing to the discussion.

In all cases the culture solution at the beginning of the experiments was 0.01 molal with respect to potassium, calcium, magnesium, and sulphate, and 0.03 molal with respect to nitrate. This concentration was not unduly changed in consequence of the growth of the plants. Phosphate was maintained by use of amounts of carefully standardized KH_2PO_4 indicated by analysis. The pH was maintained by use daily of normal solutions of potassium hydroxide and sulphuric acid. Iron was added daily, using 2 cc. of 0.5 per cent. ferric tartrate per culture. One part per million of manganese and boron were added to the solutions at the beginning of each experiment.

Fine healthy plants were produced under all of these conditions. In the presence of 50 ppm. PO_4 at pH 7.0 slight chlorosis was noted, but apparently no untoward results occurred. At least one good head was produced per plant in each treatment and it was significant that, while about three head-bearing stalks were produced at 1 ppm. PO_4 or above, practically all plants were limited to one head-bearing tiller at lower concentrations. Also, at these lower concentrations the roots were long and spindling and were discolored, suggesting a deposit of iron oxide. In other cultures the roots were strong and lustrous.

The expressed sap

Having sufficient green material available it was decided to make a study of the expressed sap of the wheat plants grown under these carefully controlled conditions. After taking the green weights, half of the plants were systematically selected and cut in suitable lengths for handling. The material was bottled, "killed" by freezing at $-15°$ C., and the expressed sap obtained under a mechanical pressure of 200 kilograms per square centimeter immediately after thawing.[2] This method has been used successfully by many investigators among whom may be mentioned Newton (**10**) and Hoagland and Martin (**8**) of this laboratory.

Immediately after the pressure was released the sap was filtered and titrated electrometrically against 0.1 N · H_2SO_4 and 0.1 N · NaOH, using the hydrogen electrode and converting the E.M.F. readings to pH by use of

[2] The author is indebted to the Division of Pomology, University of California, for the use of the cold chambers and press required for this operation.

TABLE VI

EXPERIMENTS 1, 2, AND 3

DETAILS CONCERNING THE GROWTH AND COMPOSITION OF WHEAT PLANTS GROWN IN FLOWING CULTURE SOLUTION

CULTURE NO.	EXPERIMENT 1				EXPERIMENT 2			EXPERIMENT 3			
	1 (CONTROL)	2	3	4	5	6	7 (SUB-CONTROL)	8	9	10	11 (SUB-CONTROL)
Culture solution, pH	5.5	7.0	5.5	7.0	5.5	5.5	5.5	5.5	7.0	5.5	5.5
PO_4 ppm.	5.0	5.0	50.0	50.0	0.05	0.5	5.0	0.1	0.1	1.0	5.0
Date of germination	June 7	June 7	June 7	June 7	July 17	July 17	July 17	Sept. 1	Sept. 1	Sept. 1	Sept. 1
Date of planting	June 14	June 14	June 14	June 14	July 24	July 24	July 24	Sept. 7	Sept. 8	Sept. 8	Sept. 8
Date of harvest	July 23	July 23	July 23	July 23	Sept. 8	Sept. 8	Sept. 8	Oct. 16	Oct. 18	Oct. 18	Oct. 21
Age (days)	39.	39.	39.	39.	46.	46.	46.	38.	40.	40.	43.
Tops, dry weight per plant (gm.)	2.08	1.56	1.95	2.11	0.74	0.76	1.86	0.87	0.83	1.93	2.36
Moisture, per cent.	83.7	84.7	84.0	83.3	82.9	86.2	83.9	79.4	80.8	83.5	83.2
PO_4, per cent.	2.26	2.17	2.26	2.17	1.19	2.90	2.49	0.68	0.87	2.40	2.53
PO_4 absorbed per plant (mg.)	47.0	33.8	44.1	45.7	8.8	22.0	46.3	5.92	7.22	46.3	59.6
Roots, dry weight per plant (gm.)	0.29	0.25	0.28	0.26	0.16	0.10	0.29	0.26	0.23	0.29	0.34
PO_4 per cent.	2.35	3.18	3.88	3.88	1.15	2.62	3.60	0.70	0.73	2.52	3.32
PO_4 absorbed per plant (mg.)	6.81	7.95	10.9	10.1	1.84	2.62	10.4	1.82	1.68	7.30	11.3
Relative absorption of phosphate											
In entire plant (c.f. 1)	100.	77.8	102.	104.	19.7	45.7	105.	14.3	16.5	100.	132.
In tops only	100.	72.	94.	97.	18.7	47.	98.5	12.6	15.4	98.5	127.
In roots only	100.	117.	160.	148.	27.	38.5	154.	26.8	24.7	107.	166.
No. of tillers per plant	5.0	3.9	3.7	4.4	2.2	2.6	3.8	1.4	1.9	4.2	4.2
No. head-bearing stalks per plant	3.7	3.1	3.5	?	1.0	1.0	3.0	1.2	1.1	2.9	2.7

the tables compiled by SCHMIDT and HOAGLAND (**14**). Ten cubic centimeters of the sap, diluted with 10 cc. CO_2-free distilled water, served for each titration. Other portions of the sap were analyzed for phosphate (volumetrically and colorimetrically), and also for other ions where possible. The results of the titrations are expressed as curves in figs. 2, 3, and 4, and the analytical data are reported in table VII.

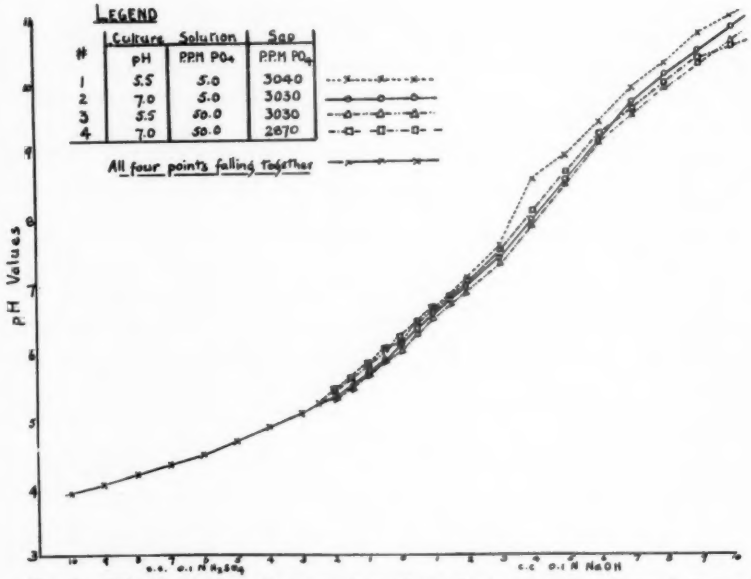


FIG. 2. Titration curves of expressed sap from plants grown in cultures 1, 2, 3, and 4, experiment 1. Between pH 5.3 and pH 3.9 the points so nearly coincided that it was necessary to use a single curve to represent the results, and avoid a confusion of lines.

The results are instructive in showing that the reaction of the sap obtained by these methods is not materially altered by phosphatic treatment or reaction of the culture solution in the range used. The buffer action of the sap, as determined by electrometric titration of the usual type, is markedly influenced, however, on the alkaline side but not on the acid side. This difference may be correlated with the amount of phosphate in the cell sap.

The phosphate of this sap is related to the phosphate of the culture solution, but there seems to be no direct proportionality. The results indicate that the phosphate of the sap is approximately constant when the medium contains between 0.5 ppm. PO_4 and 50 ppm. PO_4. When the culture solution is more dilute with respect to phosphate the sap seems relatively

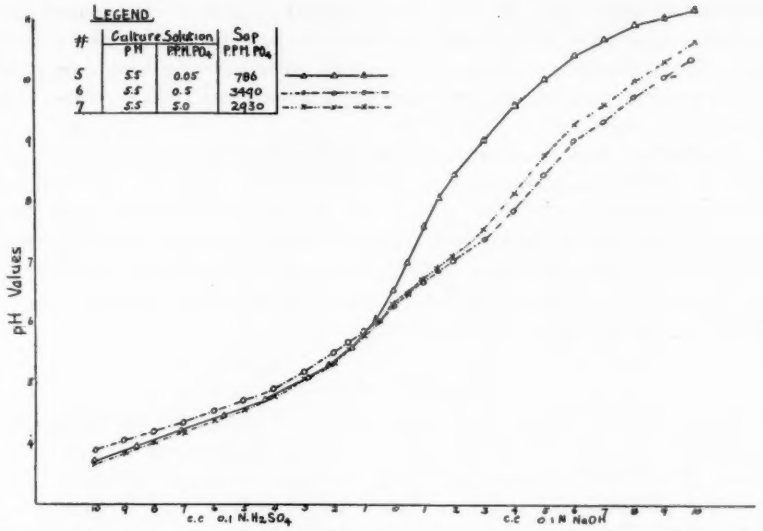

LEGEND
| Culture Solution pH | Culture Solution P.P.M. PO_4 | Sap P.P.M. PO_4
5 | 5.5 | 0.05 | 786
6 | 5.5 | 0.5 | 3490
7 | 5.5 | 5.0 | 2930
pH Values
c.c 0.1 N. H_2SO_4
c.c 0.1 N NaOH

FIG. 3. Titration curves of expressed sap from plants grown in cultures 5, 6, and 7, experiment 2.

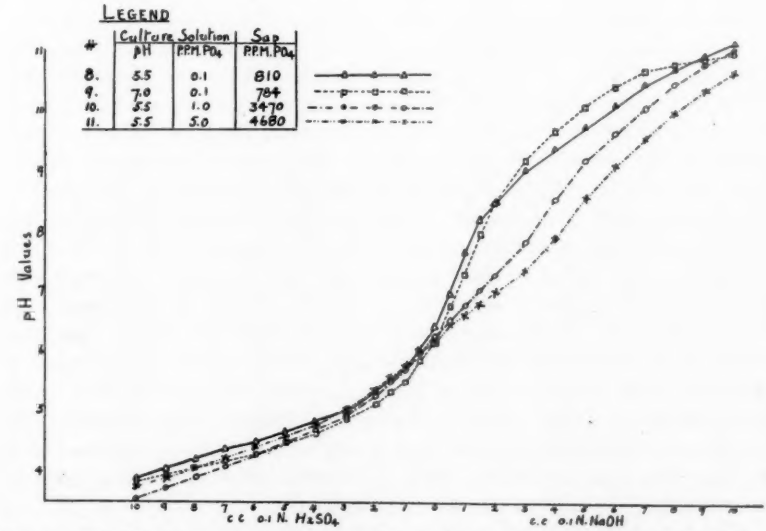

LEGEND
| Culture Solution pH | Culture Solution P.P.M. PO_4 | Sap P.P.M. PO_4
8. | 5.5 | 0.1 | 810
9. | 7.0 | 0.1 | 784
10. | 5.5 | 1.0 | 3470
11. | 5.5 | 5.0 | 4680
pH Values
c.c 0.1 N. H_2SO_4
c.c 0.1 N. NaOH

FIG. 4. Titration curves of expressed sap from plants grown in cultures 8, 9, 10, and 11, experiment 3.

constant at about 800 ppm. PO_4. The non-sap phosphate of the plant does not seem to be related to the concentration of the culture solution. Again, the close approximation of volumetric and colorimetric methods in practically all cases *suggests* that the sap phosphate is inorganic in nature, or is very loosely combined.

A relatively complete analysis of the sap from cultures 10 and 11, experiment 3, enables a calculation of the reaction values. These results indicate a considerable excess of base, suggesting that an organic anion must play an important part in the sap of the plant. What part this anion may contribute to the buffer system of the plant is a matter of conjecture at present, but it seems probable that phosphate plays an important rôle in counteracting any change on the alkaline side.

Discussion of results

It is unfortunate that the results reported in this paper are too meager in quantity to allow the formulation of definite conclusions or generalizations. Much further study, involving the use of many different varieties of wheat and different types of plants, and a more extensive exploration of the effects of lower concentrations of phosphate, will be necessary before these ends are attained. The method of attack seems worthy of mention to investigators in plant physiology and it is hoped that the suggestions obtainable from the results may assist in future researches.

As yet no one has been able to solve the problem of the phosphate nutrition of plants. That phosphate is absorbed from very dilute solutions in the soil is recognized, so it will be necessary to explore a similar range of concentration in the study of the question from a natural aspect. Obviously the use of solutions supplying phosphate at the rate of several hundred parts per million obscures the problem rather than elucidates it. In soil cultures it is impossible to evaluate any action of the roots on the solid phase, so the investigator must resort to solution cultures to obtain information concerning minimal concentrations and optimal concentrations. In these experiments the soil cultures proved valuable in indicating that, in the neutral soils used, the composition of the displaced solution gave the approximate composition of the physiological soil solution with respect to phosphate, and also in indicating the range of concentration which it would be profitable to study. Other investigators, PARKER and TIDMORE (**11**), VON WRANGELL and HAASE (**17**), and a host of others, have published data showing that soils in other parts of the world afford a similar range in concentration of phosphate in the soil solution.

The data here reported, obtained from both soil and solution cultures, indicate that one part per million of phosphate in the soil solution, if main-

TABLE VII

EXPERIMENTS 1, 2, AND 3

FLOWING SOLUTION CULTURES. ANALYSIS OF SAP EXPRESSED FROM TOPS OF WHEAT PLANTS GROWN IN CONSTANTLY FLOWING SOLUTION, AFTER FREEZING AT −15° C. TO −20° C. TO "KILL" THE TISSUE

CULTURE NUMBER	EXPERIMENT 1 (June 14th to July 23rd)				EXPERIMENT 2 (June 24th to September 8th)			EXPERIMENT 3 (September 8th to October 21st)			
	1 (CONTROL)	2	3	4	5	6	7	8	9	10	11
Culture solution pH.	5.5	7.0	5.5	7.0	5.5	5.5	5.5	5.5	7.0	5.5	5.5
PO_4 ppm.	5.0	5.0	50.0	50.0	0.05	0.5	5.0	0.1	0.1	1.0	5.0
Sap analysis pH	6.26	6.18	6.13	6.28	6.54	6.30	6.29	6.40	6.24	6.29	6.20
PO_4 (color) ppm.	2940.	2940.	3030.	2780.	800.	3300.	2800.	N.D	570.	2700.	3450.
PO_4 (total) ppm.	3040.	3030.	3030.	2870.	786.	3490.	2930.	810.	784.	3470.	4630.
SO_4 ppm.	.....	.....	.....	.....	.....	.....	.....	1070.	3670.*	890.	1370.
NO_3 ppm.	.....	.....	.....	.....	.....	.....	.....	N.D.	N.D.	5000.	5300.
Ca ppm.	.....	.....	.....	.....	720.	800.	673.	760.	1130.	715.	665.
Mg ppm.	.....	.....	.....	.....	700.	560.	645.	670.	980.	580.	590.
K ppm.	.....	.....	.....	.....	.....	.....	.....	.....	.....	6200.	7600.
Fe ppm.	.....	.....	.....	.....	.....	.....	.....	35.	43.	20.	19.
Ratio, moisture: dry matter	5.1	5.5	5.3	5.0	4.8	6.2	5.2	3.9	4.2	5.1	5.0
Sap phosphates as per cent. dry matter	1.55	1.66	1.60	1.49	0.38	2.16	1.52	0.32	0.33	1.77	2.32
Total phosphate as per cent. dry matter	2.26	2.17	2.26	2.17	1.19	2.90	2.49	0.68	0.87	2.40	2.53
Non-sap phosphate as per cent. dry matter	0.71	0.51	0.66	0.68	0.81	0.74	0.97	0.36	0.54	0.63	0.21
Ratio, sap phosphate: total phosphate	0.69	0.77	0.71	0.69	0.32	0.74	0.61	0.47	0.38	0.74	0.92

tained, is adequate for the growth of Pusa 4 wheat under the conditions of the experiments.

A comparison of the results of soil and solution cultures (tables IV and VI) leads to the conclusion that, in these neutral, highly buffered soils, the displaced solution represents the physiological soil solution. Apparently the carbonic acid of the roots is neutralized immediately by calcium carbonate or other reactive components of the solid phase of the soil, preventing a change in the reaction sufficient to affect the solubility of the soil phosphates. This conclusion is supported by the results obtained from soil 33 in comparison with soil 33a (table IV). The addition of a considerable quantity of very finely ground rock phosphate which did not affect the soil solution appreciably, did not increase the absorption of phosphate by the wheat plants. Had carbonic acid played any appreciable part under these conditions the absorption would have been considerably augmented.

In the soil cultures, calcium in the soil solution is recognized as an agent active in the *retardation* of the solution of solid phase phosphate under a vigorously growing crop (table V). However, to account for the range in phosphate concentration in different soil types of the same reaction, it is necessary to consider other factors besides the concentration of calcium in the soil solution. That the nature of the solid phase phosphate is very important is indicated by the behavior of soil 33 (table III). The added phosphate was largely precipitated to form a solid phase compound yielding the same concentration of phosphate in the soil solution with or without a growing crop; and this concentration could not in any way be related to the concentration of phosphate in the original soil solution. This also did not vary under the action of a heavy crop.

It is permissible to study the results of the solution culture experimentation in greater detail. It must be admitted at the outset that the plants receiving 0.5 ppm. PO_4, culture 6, experiment 2, table VI, were spindling and made growth similar in amounts to those plants receiving 0.05 ppm. PO_4 and 0.1 ppm. PO_4. The composition, however, was comparable with the controls receiving 5.0 ppm. PO_4. No explanation is apparent at present. Further experimentation is necessary in this range of concentration between 0.1 ppm. PO_4 and 1.0 ppm. PO_4.

Other cultures indicated the same conclusion as did the soil cultures and substantiated the conclusion of SCHLOESING fils (**13**) referred to above. Below 1.0 ppm. PO_4 in the culture solution the absorption of phosphate by these plants was seriously affected. The composition (with respect to phosphate) of the expressed sap and the dry matter of the plants grown in solutions containing less than 0.5 ppm. PO_4 differed greatly from that of others receiving the more liberal concentrations. And the composition of

the plants, expressed in these terms, appeared to be approximately unaffected by the composition of the culture solution (with respect to phosphate) between 0.5 ppm. PO_4 and 50 ppm. PO_4 for the high level and between 0.5 ppm. PO_4 and 0.1 ppm. PO_4 for the low level. As regards total absorption per plant by the tops the same relationship holds except that 0.5 ppm. PO_4 in the culture solution allowed an absorption of phosphate intermediate between these groups.

What does this mean?

It seems apparent that the composition of the plant is affected by the composition of the soil solution, or culture solution, but there is no direct proportionality. Doubling the concentration in the solution does not cause a proportionate increase in absorption as 0.05 ppm. PO_4 gave results comparable with 0.1 ppm. PO_4. More emphatic is the fact that 5.0 ppm. PO_4 (and even 1.0 ppm. PO_4) gave results strictly comparable with 50 ppm. PO_4 when one considers the amount of growth and tillering, the total phosphate absorbed, the percentage composition of the dry material, the composition of the expressed sap with respect to phosphate, and the buffer qualities of the sap. If there is a range of direct proportionality it is suggested that it will be limited to a relatively narrow range between 0.1 ppm. PO_4 and 1.0 ppm. PO_4 in the culture solution. It is significant that this is a range very common to the soil solution and is deserving of further attention.

Contributing factors of great importance need consideration and control. Varietal differences provide a field for exploitation and it is very possible that the results reported in this paper would be somewhat different if other varieties were used.

Different types of plants exist and are physiologically different in their behavior in soils. Will these differences be as apparent in solution cultures? Seasonal differences cannot be eliminated on a large scale at present. In this investigation similar controls were used in all three experiments involving the solution culture and a study of table VI shows that growth, the absorption, and the time taken to reach a given stage of maturity are dependent in some degree on seasonal conditions.

The question of the effect of phosphate on the "earliness" of wheat is raised by the results of the solution culture experiments. It is generally accepted that phosphate hastens maturity under field conditions. The figures in table VI do not support the field results for some reasons not yet understood. Various possibilities may be mentioned among which are varietal differences; greenhouse conditions *may* not be comparable with field conditions; the action of the phosphate in the field may be indirect and due to the changed ***fertility*** of the soil.

Criticism may be levelled at these experiments on account of the period of harvesting. Field workers agree that with cereals the period of "flowering" is the most reliable index of the period of ripening and is much easier to determine than is the stage called ripeness. The work of WIEBE (**16**) with oats may be cited in support of this contention.

Summary

1. Wheat plants were grown in soil cultures and in flowing solution cultures in an attempt to study the absorption of phosphate.

2. The composition of the soil solution, especially with regard to phosphate, was determined by analysis of the displaced solution. In the several soils studied the concentration varied from 0.15 ppm. PO_4 to about 10 ppm. PO_4. The behavior of the phosphate under the influence of a crop of wheat was studied by comparison with uncropped controls.

3. Flowing solution cultures were used to maintain the concentration of phosphate. Concentrations from 0.05 ppm. PO_4 to 50 ppm. PO_4 were studied in a series of three experiments.

4. While the absorption of phosphate was affected by the concentration of the soil or culture solution with respect to phosphate, there was no direct proportionality.

5. The data indicate that there were two categories under the conditions of these experiments:

(a) Vigorous growth with tillering and high absorption of phosphate between 1.0 ppm. PO_4 and 50 ppm. PO_4 in the culture solution.

(b) Small growth with greatly reduced tillering and low absorption of phosphate between 0.05 ppm. PO_4 and 0.1 ppm. PO_4 in the culture solution.

The amount of growth of tillering, and quantity of phosphate absorbed by the plants in each group were remarkably similar.

6. If a region of direct proportionality exist, it is suggested that it will be in the range between 0.1 ppm. PO_4, and 1.0 ppm. PO_4—the range most commonly met with in soil solutions.

7. The composition and buffer qualities of the expressed sap were reflected by the total absorption of phosphate. Phosphate was effective in buffering against alkali, but the concentration of phosphate had no observable effect against acid. An excess of base in the expressed sap suggested the presence of an organic anion.

8. The non-sap phosphate of the plant showed no proportionality to the concentration of phosphate in the culture solution or in the expressed sap.

9. The displaced solution of the soils studied, neutral and highly buffered in character, represented the physiological soil solution with respect

to wheat. In these soils the concentration of phosphate in the soil solution was dependent on the nature of the solid phase rather than on the composition of the soil solution.

DEPARTMENT OF AGRICULTURE,
PERTH, WESTERN AUSTRALIA.

LITERATURE CITED

1. ANDERSSEN, F. G. A device for maintaining constant level of culture solutions. Plant Physiol. **1**: 417–418. 1926.
2. ATKINS, W. R. G. The rapid determination of available phosphate in soil by the coeruleo-molybdate reaction of Denigés. Jour. Agr. Sci. **14**: 192–197. 1924.
3. BEYER, H. Bericht über die im Sommer 1867 an der Versuchs-Station Regenwalde ausgeführten Wasserculturversuche. Landw. Vers. Stat. 11: 262–287. 1869.
4. BIRNER, DR., and LUCANUS, DR. Wasserculturversuche mit Hafer. Landw. Vers. Stat. **8**: 128–177. 1866.
5. BREAZEALE, J. F., and BURGESS, P. S. The availability of phosphate in calcareous or alkaline soils. Arizona Agr. Exp. Sta. Tech. Bull. 10. 1926.
6. BURD, J. S., and MARTIN, J. C. Water displacement of soils and the soil solution. Jour. Agr. Sci. **13**: 265–295. 1923.
7. HOAGLAND, D. R., and MARTIN, J. C. A comparison of sand and solution cultures with soils as media for plant growth. Soil Sci. **16**: 367–388. 1923.
8. ————————. Effect of salts on the intake of inorganic elements and on the buffer system of the plant. Calif. Agr. Exp. Sta. Tech. Paper 8. 1923.
9. KOSSOWITCH, P. Le rôle des plantes dans la dissolution des principes nutritifs du sol qui se trouvent à l'état nondissous. Ann. de la Sci. Agron. 2nd series, I: 220–245. 1903.
10. NEWTON, J. D. A comparison of the absorption of inorganic elements, and of the buffer systems of legumes and non-legumes, and its bearing upon existing theories. Soil Sci. **15**: 181–204. 1923.
11. PARKER, F. W., and TIDMORE, J. W. The influence of lime and phosphatic fertilizers on the phosphorus content of the soil solution and of soil extracts. Soil Sci. **21**: 425–441. 1926.
12. PRIANISCHNIKOW, D. Über den Einfluss von kohlensäuren Kalk auf die Wirkung von verschiedenen Phosphaten. Landw. Vers. Stat. **75**: 357–376. 1911.

13. SCHLOESING fils, TH. Etude sur l'acide phosphorique dissous par les eaux du sol. Ann. de la Sci. Agron. **1**: 316–359. 1899.
14. SCHMIDT, C. L. A., and HOAGLAND, D. R. Table of pH, H^+ and OH^- values corresponding to electromotive forces determined in hydrogen electrode measurements, with a bibliography. Univ. of California, Pub. in Physiology, **5**: 23–69. 1919.
15. TEAKLE, L. J. H. Phosphate in the soil solution as affected by reaction and cation concentration. Soil Sci. **25**: 143–162. 1928.
16. WIEBE, G. A. Inheritance of characters in a barley and an oat cross. Master's Thesis, Div. Genetics, Univ. of California. 1925.
17. VON WRANGELL, M., and HAASE, W. Über den Phosphorsäuregehalt natürlicher Bodenlösungen. Landw. Jahrb. (Sonderabdruck. Zeitschr. f. wissenschaft. Landwirtsch). **63**: 707–738. 1926.

THE EXTRACTION OF NITROGENOUS MATERIALS FROM PEAR TISSUES

F. B. Lincoln and A. S. Mulay

Three problems have been investigated in connection with removing the nitrogenous compounds from pear tissues: first, a suitable method for preservation of tissues previous to such an extraction; second, methods for obtaining an aqueous extract; third, the nitrogenous fractions found in expressed sap. In general the total N extracted has been divided into proteins and non-proteins, which because of the lack of exact separation are referred to as "proteins" and "non-proteins." Apple and spinach tissues were also included in the investigation for the purpose of comparison. As it was not possible to demonstrate the presence of nitrates in such pear tissues as were used, the simple Gunning method for the determination of total nitrogen of the A. O. A. C. was used in all cases. For the sake of brevity reference has been made to previous work in this field only when it seemed necessary.

I. The influence of preservation on distribution and extractability of nitrogenous materials of pear tissues

The treatments investigated were of two types: (1) those which kill—such as desiccation, freezing, and the use of chemical cytolizing agents; (2) treatments permitting the tissues to remain alive, such as moderately low temperatures. Besides these treatments, which were intended to minimize chemical change, others were tried which were considered not to be so favorable. These latter were intended to show the influence of two disturbing factors, autolysis, and heat coagulation of the proteins. The several maltreatments used were the storage of minced tissues, the incubation of such tissues, and the exposure to coagulating temperatures during desiccation.

Materials and methods

The leaves used were of the Beurre Hardy variety collected in the early summer. The other tissues were bark and wood from the basal portion of vigorous-growing Bartlett shoots. The investigation covered a period of two years. The second year the same tissues were used but the N content of all was higher because the samples were taken from younger trees. The leaves for 1928 appeared to contain more N because the petioles were not included. Usually bark and wood were separated from each other at the beginning of the various treatments. In one case the twigs were frozen with the bark on the wood, and the separation made after thawing.

Desiccation

The general method employed was to subject a sample of tissue to some preservation treatment and then to measure the influence of the treatment by its effect on the "non-proteins" and the total extractable nitrogen. The procedure followed in obtaining these fractions will be reported later in this paper.

Samples were dried at two temperatures, 50° and 85° C. These temperatures were chosen because Thomas (7) and others had found the former

TABLE I

Pear leaves, 1927

All values are expressed as per cent. of total N in the sample

Total nitrogen = 1.94 per cent. of dry weight

Lot	Treatment	Total N in filtrate "non-protein"	Total N in iron precipitate "proteins"	Total N extracted
		per cent.	*per cent.*	*per cent.*
L 1	Fresh	3.4	25.5	28.9
	Fresh	3.8	29.0	32.8
L 2	Dried 50° C.	5.05	22.1	27.15
	Dried 50° C.	4.4	25.6	30.0
L 3	0° C. 1 week	3.18	29.8	32.98
L 4	4–5° C. 1 week	3.26	30.2	33.46
L 5	4–5° C. 4 weeks	6.58	30.4	36.98
L 6	7° C. 2 weeks	3.48	21.1	24.58
L 7	4–5° C. 1 week (minced)	2.48	15.15	17.63
L 8	Minced, kept at room temp. for 24 hours and then dried at 50° C.	5.15	14.9	20.05
L 9	−23° C. 8 weeks	3.6	28.9	32.5
L10	Frozen (liquid air)	2.57	16.9	19.47
L11	Ether	3.16	10.12	13.28
L12	Incubated 37° C. 4 days	4.86	7.84	12.75

Pear leaves, 1928

Total nitrogen = 2.808 per cent. of dry weight

		per cent.	*per cent.*	*per cent.*
L13	Fresh	3.4	37.58	40.98
L14	Minced. Incubated 24 hrs.	3.4	20.7	24.10
L15	Dried at 50° C.	5.0	42.5	47.50
L16	Dried at 85° C.	3.58	3.50	7.08

to be quite satisfactory, and the latter was supposed to be high enough to show the injurious effects of temperature. Fan ventilated ovens were used and even at 50° C. most of the moisture was removed in the first three hours. It may be stated that moisture determinations made at 50° C. on these materials were found quite reliable; with an increase in temperature to 100° only about an additional 1 per cent. of moisture was given off.

From the data presented in tables I, II, and III, the influence of drying at 50° C. may be seen by comparing the data from desiccated and fresh samples (L2, L15, B2, B11, W2, W12). In leaves, drying increased the filtrate nitrogen, the "protein" extracted being about the same as that

TABLE II

BARK, 1927

ALL VALUES ARE EXPRESSED AS PER CENT. OF TOTAL N IN THE SAMPLE

TOTAL NITROGEN = 0.8008 PER CENT. OF DRY WEIGHT

LOT	TREATMENT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN IRON PRECIPITATE "PROTEINS"	TOTAL N EXTRACTED
		per cent.	*per cent.*	*per cent.*
B 1	Fresh	8.63	11.15	19.78
B 2	Dried at 50° C.	8.73	16.35	25.08
B 3	−23° C. 1 week	7.1	8.63	15.73
B 4	−23° C. 1 week (unseparated from wood)	10.29	10.29	20.58

BARK, 1928

TOTAL NITROGEN = 1.022 PER CENT. OF DRY WEIGHT

		per cent.	*per cent.*	*per cent.*
B 5	Fresh	8.55	17.35	25.9
B 6	Frozen and thawed 24 hrs.	10.12	13.78	23.9
B 7	Unseparated from wood frozen	13.8	21.6	35.4
B 8	Ether	7.3	15.8	23.1
B 9	Minced and kept for 24 hours, 20° C.	5.8	21.3	27.1
B10	Minced and kept for 24 hours, 20° C., then dried at 50° C.	4.64	1.38	6.02
B11	Dried at 50° C.	8.6	22.1	30.7
B12	Dried at 85° C.	9.54	9.86	19.4

TABLE III

WOOD, 1927

ALL VALUES ARE EXPRESSED AS PER CENT. OF TOTAL N IN THE SAMPLE
TOTAL NITROGEN = 0.539 PER CENT. OF DRY WEIGHT

LOT	TREATMENT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN IRON PRECIPITATE "PROTEINS"	TOTAL N EXTRACTED
		per cent.	*per cent.*	*per cent.*
W 1	Fresh	14.5	8.63	23.13
W 2	Dried at 50° C.	15.65	8.00	23.65
W 3	−23° C. 1 week	15.2	10.63	25.83
W 4	−23° C. 1 week (unseparated)	16.6	10.4	27.00
W 5	Minced—incubated at 37° C. for 4 days	18.85	11.1	29.95

WOOD, 1928

TOTAL NITROGEN = 0.868 PER CENT. OF DRY WEIGHT

		per cent.	*per cent.*	*per cent.*
W 6	Fresh	16.2	12.2	28.4
W 7	Frozen and thawed for 24 hours	17.4	18.4	35.8
W 8	Frozen unseparated from bark	17.1	20.0	37.1
W 9	Ether	16.4	19.7	36.1
W10	Minced and kept 24 hours at 20° C.	10.9	19.7	30.6
W11	Minced and kept 24 hours at 20° C. then dried at 50° C.	12.5	3.0	15.5
W12	Dried at 50° C.	16.4	17.8	34.2
W13	Dried at 85° C.	15.3	11.1	26.4

from the fresh sample. In wood the difference was not so marked, and in bark the yields were about the same for both treatments. The results showed that drying bark and wood at 50° C. was very satisfactory. Why this treatment apparently caused hydrolysis of proteins in the leaves, as evidenced by the increase in the "non-protein" fraction, is not known.

The temperature of 85° C. (L16, B12, W13), as was expected, reduced the amount of "protein" extracted. This effect was evident in all the extracts, with a striking reduction in the leaf and bark samples. The higher temperature seemed to give a truer picture of the "non-protein" N in leaves than did drying at 50° C. while the same fractions in bark and wood were influenced slightly in the direction opposite to each other.

The use of 85° may have its place in experiments where only total N and "non-protein" fractions are under consideration. It has the advantage that killing and dehydration are more rapid.

Desiccation must have favor in cases of long periods of storage and, what is also very important, dried samples of wood tissues allow easier disintegration by grinding, and also permit the use of sieves for the grading of the ground materials.

Freezing

Freezing is often a very convenient method for rapidly limiting chemical changes, particularly when samples are to have juices expressed from them. The samples in this experiment were placed in glass containers and were stored for varying lengths of time at −27° C. in a cold storage room. In this way leaves were kept in an unaltered condition for 8 weeks (L9). They retained their green color until the sample was thawed. Bark and wood held at this temperature for one week showed only slight changes (B3, W3). Freezing may have a disadvantage in that the tissue is left dead and at the same time full of water, which may permit many changes to take place upon thawing. Nightingale, Robbins, and Schermerhorn (5) report changes in nitrogen fractions of sweet potato roots due to thawing at 2° C. for 12 hours. To get the full effect of the possible changes during and following thawing, wood and bark samples which had been frozen were allowed to stand in their containers at room temperature for 24 hours before they were ground and extracted (B6, W7). While this treatment is extreme, the changes effected in the N fraction here determined were surprisingly small in the wood, but in the bark some hydrolysis had occurred, yet not so much as was expected.

Delaying the separation of bark from the wood until after thawing of the tissues was tried both years, with similar results (B4, B7, W4, W8), an increase in all nitrogen fractions. These results appear to be similar to those obtained with frozen samples subjected to a long period of thawing (B6, W7) rather than to separate samples of frozen bark and wood, which were ground and extracted without a long period of thawing. As the time required for the separation of the bark from the wood of the frozen samples was very short, there is no explanation to offer for this resemblance. During freezing and thawing there is a movement of water out of the sample to its surface and then back again to the interior. How much this translocation of water is accompanied by the movement of solutes is not known.

Cold non-freezing temperatures

Moderately cold temperatures, 0° C. to 7° C., preserved fresh leaves well for a week or two (L3, L4, L6). Holding at 4° C. (L5) for a month resulted in an increase of "non-protein" N. Even after this long period

the sample was free from mold and had retained its full green color. Preservation at these temperatures has the advantage of keeping the tissue alive and at the same time greatly reducing chemical changes.

Chemical reagents

Ether has long been used to make cells permeable, so that their liquid contents could be more easily extracted. This reagent seemed to affect the extractable protein fraction of different tissues differently; there was a marked reduction in leaves (L11), slight reductions in bark (B8), and an increase in the wood (W9). In the same samples ether treatment did not greatly influence the "non-protein" fraction although there may have been a reduction of it in the bark. The ether treatment was not exactly alike in the various samples. The leaves were immersed in ether, while with bark and wood 25 ml. of ether was placed in a quart jar and only the vapor came in contact with most of the tissue.

Liquid air has been effective in other studies in making tissues permeable. In this experiment (L10) it reduced both nitrogen fractions in the treated leaves.

Maltreatments

El Sawy (**3**) has reported the effects of injury to pear tissues resulting from cutting shoots into short lengths. The effect was a rapid hydrolysis of polysaccharides accompanied by an increase in respiration. In the present investigation several treatments were made in which the tissues were minced in a food chopper. The minced tissues were then kept at room temperature for a day in closed glass containers. As a result of this severe treatment leaves (L14) showed a decrease in extractable "proteins." Bark and wood (B9, W10) acted alike, but contrary to leaves; they showed a decrease in the "non-protein" fraction and an increase in proteins. When portions of the minced tissue were dried at 50° C. (L8, B10, W11) there was a great reduction of extractable "protein" N.

A sample of minced leaves was kept for a week at 4° C. (L7) resulting in a marked reduction of both fractions of extractable N. It is not clear why injury should so often be accompanied by a reduction in "non-protein" N. This sample was tested for free ammonia and no volatile alkali was found.

When leaves and wood were minced and suspended in water, covered with toluol, and incubated at 37° C. for 4 days, some slight hydrolysis took place (L12, W5). This change was very much less than was expected, assuming that the conditions were quite favorable for autolysis. These experiments, taken with those where a long period of thawing was used (B6, W7), give some idea of the effects of enzymes present in the tissue when allowed to act for a moderate period. They are very feeble when

compared with the effects of added animal enzymes. Some data showing the effect of enzymes present in leaf tissue are given by CHIBNALL (1). From these data it appears that their action may last for only a short time.

The effect of maltreatment by overheating of "proteins" is found in the samples dehydrated at 85° C. With leaves and bark (L16, B12) the "protein" fraction was markedly reduced in extracts of heated samples.

APPLE TISSUES

Apple tissues of McIntosh variety were collected in the middle of the summer and subjected to the treatment while fresh. The results are found in table IV. It will be seen that the N fractions extracted from the different tissues are in the same relation to each other as they are in the extracts of pear tissues. The striking difference is that much more of the N of apple tissues is extracted from each of the various tissues.

TABLE IV

APPLE TISSUES

ALL VALUES ARE EXPRESSED AS PER CENT. OF TOTAL N IN THE SAMPLES

TREATMENT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN IRON PRECIPITATE "PROTEINS"	TOTAL N IN EXTRACT
	per cent.	*per cent.*	*per cent.*
Fresh leaves	6.65	36.65	43.3
Fresh bark	12.12	32.78	44.9
Fresh wood	35.9	13.7	49.6

When preservation must be resorted to there are several procedures which will give results quite similar to fresh tissues. These are dehydration at 50–55° C., storage at 0° C. or a few degrees above for short periods, or freezing when a longer storage period is necessary. If the "protein" fraction is not to be extracted, drying at temperatures of 80–85° C. will stop changes rapidly. Samples should be preserved with as little mincing as possible since injured tissue may change rapidly.

How best to preserve plant material for chemical analysis is still an open question. The answer to this question depends somewhat on the investigation and the equipment available for it. Facilitation of work may be of more importance than extreme exactness; and if fresh material can not be used, circumstances will determine the method of preservation. Thus, if storage is to be for a long time or if firm tissues must be disintegrated, drying is likely to be the best method. Low temperatures have their place where sap concentrations are of chief interest. It should be

remembered that different parts of a plant may not all preserve equally well by a given method and also that any organ may so change its composition or nature during the season that the same method may not give equally reliable results at all times. The present study aims to show the types and magnitudes of changes that may be introduced by preservation, and will help to determine the dependability of results obtained by the different methods.

II. Methods for the extraction of cytoplasmic nitrogen

Generally only 10–25 per cent. of the total N of pear tissues is soluble in the expressed sap, and is almost wholly in a "non-protein" form. The present study was undertaken to see how much of the remaining insoluble N could be made soluble or peptized in water. The procedure was to extract the very finely divided tissue with water containing different ions, or to hydrolyze with acid or animal enzymes. There are at least three disturbing factors to such extractions: (1) the acidity of the tissue, (2) naturally contained tannins, and (3) the inability to get the tissues absolutely disintegrated. The latter is best accomplished by drying the tissues at 50° C., grinding them coarsely in a mill and completing the grinding to a powder in a ball mill. When such leaf powder was examined under the microscope it was found that most of the cells were completely broken apart. Only a few bits of tissue of several cells each remained; these were portions of the harder tissues composed of smaller cells.

There is considerable uncertainty regarding the action of the tannins present in pear tissues. They seem to be present; for expressed sap will cause precipitation in a gelatin solution, and will also give the color reactions of tannins. However, when tannic acid was added to water extracts of pear tissues, table VIII, it did not increase the amount of N in the precipitate over the amount caused by the acid used to adjust the reaction for the precipitation by tannins. Tannin-protein complexes are soluble in alkaline solutions, which may account for the peptizing effect of alkaline solutions in these studies. On the other hand, spinach, which very likely contains no tannins, responds like pear tissues when extracted with alkaline solutions. As the work proceeds and attempts are made to purify cytoplasmic proteins perhaps some evidence of a tannin residue may appear. There is also the possibility that the inherent tannins do not precipitate cytoplasmic proteins. The extracts of pear tissues were in themselves so highly colored that it was not possible to apply tannin color reactions to them. Furthermore, combined tannins would not give the test had they been present. There was also the hope that in alkaline solutions the tannins would be oxidized by the air which was forced through the solutions by the mechanical stirrer, and in their oxidized form would not combine with the

proteins when the solutions were neutralized. The solutions did become dark in color, but the fate of the tannins is not known.

Before the tissues can be subjected to a water extraction they should be disintegrated as completely as possible. This was accomplished by two methods. In the one the fresh tissue was ground with sand and water, and in the other the tissue was desiccated, ground, and then the powder extracted with water. The first method was used in investigating the various procedures for preserving tissue reported previously in this paper. This was required because some of the samples had to be handled in the succulent condition for comparison with the dehydrated ones.

The sand grinding method for fresh material is as follows: All samples, fresh, frozen, and dried, were ground with water, sodium carbonate, and sand, in a "Quaker City" corn mill. This mill is similar in principle to the Enterprise "Nixtamal" mill mentioned by several investigators. Generally the materials were minced in a food grinder or a Wiley mill before grinding with sand and sodium carbonate. The procedure was to weigh out 200 grams of the minced sample, and add to it 25 grams of sodium carbonate and 150 or more grams of sand. These were ground together with water. Grinding was most effective when the sample was kept in a non-liquid pasty mass. The higher yields of precipitated N in 1928 were the result of using less water while the samples were being disintegrated. Grinding four times in the mill was generally sufficient to reduce the materials so that about 75 per cent. of the sample would pass through a 100 mesh sieve.

When grinding was complete, the material was stirred into about 1500 ml. of water, allowed to stand for an hour, and then separated from this water by centrifuging. The solid residue was washed with water four times and the extracts combined. The extracts were treated with toluol and kept at 0° C. The time required for grinding, mixing, and centrifuging a sample was about three hours. It is believed that the amount of precipitable N extracted can be increased over the values here reported by vigorously agitating the ground material in water for 30 minutes or so before centrifuging. The residue after washing was dried at 85° C. and again ground for sampling. Total nitrogen was determined in triplicate on the extract and the residue.

The second method, using dry powder, is much more exact because the tissue can be disintegrated more completely and can be graded by the use of sieves and, wherever it can be used, it is recommended as superior to the previous method. It was used in attempts to get complete extraction of nitrogen, the results of which will be given further on in this paper. The method is essentially to suspend 10 grams of dry pulverized tissue in 250 ml.

of extracting solution, the suspension being agitated by a mechanical stirrer for two hours. At the end, the extracted tissue was collected by centrifuging. Table V gives the influence of temperature on extracting leaf powder with water. The time of these extractions was two hours.

TABLE V

EFFECTS OF TEMPERATURE ON EXTRACTION OF NITROGEN FROM LEAF POWDER IN WATER

TEMPERATURE	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN PRECIPITATE "PROTEIN"
	per cent.	*per cent.*	*per cent.*
20° C.	17.45	6.15	11.3
50° C.	14.9	6.76	8.14
80° C.	10.8	8.65	2.15

The duration of the extraction has its influence, as may be seen from table VI, which presents the results of extraction of leaf powder with water made alkaline with sodium carbonate. Two hours was the time selected for all subsequent extractions.

TABLE VI

EFFECTS OF LENGTH OF TIME OF EXTRACTION ON THE NITROGEN FRACTIONS OF LEAF POWDER + Na_2CO_3

TREATMENT	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN PRECIPITATE "PROTEIN"
	per cent.	*per cent.*	*per cent.*
2 hours, no stirring	27.80	5.2	22.6
30 minutes, stirring	43.1	5.7	37.4
2 hours, stirring	47.50	5.0	42.50
Residue from 2 hours, stirred a second 2 hours	3.0	.....	.....

PROTEIN AND NON-PROTEIN NITROGEN

While the interest here is to see how much of the ordinarily insoluble N of pear tissues could be peptized and extracted by various means, it is also worth knowing how much hydrolysis had accompanied each of the treatments. It was assumed that an increase in the "non-protein" N would be a measure of hydrolysis. This made it necessary to fractionate the N of the extracts into "protein" and "non-protein." A good discussion of the

separation of proteins from their hydrolytic products is given by THOMAS (8). He concludes that colloidal iron effects such a separation.

TABLE VII

PRECIPITATION VALUES ON FRESH LEAF AND FROZEN UNSEPARATED BARK AND WOOD, 1927, EXPRESSED AS PERCENTAGE OF TOTAL NITROGEN

Na_2CO_3 EXTRACT	TOTAL N IN EXTRACT	HEAT COAGULATION	IRON PRECIPITATE	ACID PRECIPITATE, pH 1	TOTAL PRECIPITATED	ACID pH 1 ON ORIGINAL
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Leaf	29.0	3.24	5.19	14.8	23.23	25.5
Bark	21.3	9.87	5.94	3.56	19.37	10.65
Wood	24.5	5.3	6.5	2.39	14.19	9.45

In order to find out the amount of nitrogenous compounds which was precipitated from pear extracts by various means, some experiments were made using sodium carbonate extracts from fresh leaves, from bark, and from wood. The bark and wood had been preserved by freezing and were not separated from each other until thawed. These extracts were brought to pH 5, near the isoelectric point of many proteins, by the addition of hydrochloric acid. The acidity was estimated by indicator papers. These acidified extracts were boiled and the heat-coagulated materials collected. The liquid residue was treated with colloidal iron. The precipitate formed was collected and the liquid residue was further acidified to pH 1 and a third precipitate was obtained. The results of these fractional precipitations as well as the total amount of N precipitated are reported in table VII. Portions of the original extracts were made acid to pH 1 and a precipitate was obtained, the N content of which is reported in the last section of table VII. From this data it appears that heating, or use of colloidal iron removes N compounds from the extracts of wood and bark, which acid (pH 1) alone does not remove. It may be suggested that acid alone in this case does not precipitate the heat coagulated fraction. The same appears to be true for spinach, which is discussed later. The heating of bark extracts gave a very large volume of jelly which was only freed with difficulty from its soluble forms of N.

Further experiments were carried out in 1928 in which alcohol, heating, strong acid, and tannin are compared as precipitating agents. The results are presented in table VIII.

Here the amount of N precipitated by 65 per cent. alcohol compares very well with that brought down by strong acid (pH 1). However, in leaves and wood the acid is more inclusive. To use tannic acid the extract must

TABLE VIII

PER CENT. OF TOTAL N PRECIPITATED BY HEAT, ACID, AND ALCOHOL

TISSUE	TOTAL N IN EXTRACT	ALCOHOL 65 PER CENT.	HEAT pH 6	pH 4	pH 4 + TANNIN	ACID pH 1
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Fresh wood	28.4	12.1	4.15	5.8	5.5	12.2
" bark	25.9	16.7	11.08	16.5	14.7	17.35
" leaves	40.98	22.95	8.78	11.9	8.7	37.58
" spinach	89.2	55.9	2.2			57.4
Root wood (dry)	76.5	16.2				19.4

be made more acid, pH 4, for the precipitation of proteins (7). Why the precipitation was not more complete when tannic acid was used is not

TABLE IX

INFLUENCE OF pH SERIES ON THE EXTRACTION OF NITROGEN FROM LEAF POWDER
ALL VALUES ARE IN TERMS OF PER CENT. OF TOTAL N IN THE SAMPLE

pH	REAGENT	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN PRECIPITATE "PROTEIN"
		per cent.	*per cent.*	*per cent.*
1	N/10 H_2SO_4	15.8	7.7	8.1
5	Water	17.45	5.95	11.50
6	N/10 NaOH	19.35	5.65	13.70
7	N/10 NaOH	22.4	4.6	17.8
9	N/10 NaOH	28.85	3.7	25.15
12	0.2N Na_2CO_3	47.5	5.0	42.5
13 +	2N NaOH	89.35	18.5	70.85

ROOT WOOD SERIES

INFLUENCE OF pH SERIES ON THE EXTRACTION OF NITROGEN FROM ROOT WOOD
TOTAL NITROGEN = 0.414 PER CENT. OF DRY WEIGHT
ALL VALUES ARE IN TERMS OF PER CENT. OF TOTAL N IN THE SAMPLE

pH	REAGENT	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN PRECIPITATE "PROTEIN"
		per cent.	*per cent.*	*per cent.*
1	H_2SO_4	56.4	56.4	
5	Water	38.85	38.85	
12	0.2N Na_2CO_3	57.8	56.8	1.00
13 +	2N NaOH	76.5	57.1	19.4

known. Iron is not effective without acidifying the extract, and to get a good yield the acidity must be increased to such an extent in these extracts that the acid alone becomes the precipitating agent. From the present study it appears that acid (pH 1) is a very suitable precipitating agent and probably gives a fair measure of the colloidal proteins contained in the extracts. The question as to the purity of the precipitate is left for future investigation.

Spinach has been included in this investigation in order that the results obtained with pear tissues could be compared with the results obtained by others who have worked principally with annual plants, and plants free from tannins. OSBORNE and WAKEMAN (**6**), working with a very different method of fractionation, report the colloidal proteins of spinach as being 58.7 per cent. of the total N, and the non-protein, proteose, and heat coagulatable fractions as 33.5 per cent. These figures compare very favorably with the values here obtained by the use of strong acid as a fractionating agent; these values are 57.4 per cent. as acid precipitate, and 31.8 per cent. as filtrate N (table X).

TABLE X

SPINACH SERIES

TOTAL NITROGEN = 3.56 PER CENT. OF DRY WEIGHT

TREATMENT	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN PRECIPITATE "PROTEIN"
	per cent.	*per cent.*	*per cent.*
Fresh, H_2O	89.2	31.8	57.4
Dried, H_2SO_4	42.0	42.0	
" , water	57.1	35.8	21.3
" K tartrate	48.8	34.6	14.2
" Na_2CO_3	60.5	31.9	29.0
" " (duplicate)	60.9	27.6	32.9
" NaOH	84.0	35.8	48.2
" alcohol	23.3	23.3	

CHIBNALL and GROVER (**2**) reported "non-protein" N of spinach leaves as 26.7 per cent. which is about the same as was reported by OSBORNE and WAKEMAN (**6**). In both of these cases the heat coagulable N is not included, which fact probably accounts for the percentages being lower than for the filtrate after acid precipitation (here called "non-protein" N), which is 31.8 per cent. CHIBNALL and GROVER (**2**) and OSBORNE and WAKEMAN (**6**) report 37 per cent. of the N not extracted. In the present investigation, owing perhaps to better disintegration of the sample, only about 10 per cent. of the N remained unextracted. The method used on the fresh

spinach was the one already described with the exception that no sodium carbonate was used.

The "non-protein" N fraction was increased from 33.5 per cent. to 43.6 per cent. in the investigations of OSBORNE and WAKEMAN (**6**) by drying at 60° C. In the present study the increase caused by drying was less, being from 31.8 per cent. to 35.8 per cent., but at the same time the amount of extractable "protein" was reduced about two-thirds. OSBORNE and WAKEMAN (**6**) showed no such reduction and stated that dried material was equivalent to fresh. They determined their colloidal proteins by difference rather than by actual determination. That drying is effective in decreasing extractable "proteins" is confirmed by CHIBNALL's work on Runner beans (**1**) in which air drying decreased the extractable colloidal "proteins" about one-half and at the same time the soluble "non-protein" fraction changed from 24 per cent. to 39 per cent.

INFLUENCE OF HYDROXYL ION

Having set forth and discussed the procedure for the separation of "protein" from "non-protein" materials, we shall now return to the attempt to secure a more complete extraction of nitrogenous compounds.

The influence of alkalinity on the peptization of cytoplasmic proteins is shown in table IX. Here leaf and root wood powders were extracted with water and electrolytes chosen to give different hydroxyl ion concentrations. No effort was made to buffer the solutions. The reactions reported were determined by indicator papers as far as possible, the tests being made before and after agitation. The results show that alkalinity is a most important factor in extracting "proteins" from these tissues. Table X shows that the same is true for spinach tissue. Some hydrolysis took place in all tissues with the stronger concentrations of acid and base, the hydrolysis being very marked with strong sodium hydroxide acting on leaf powder. With spinach leaves sulphuric acid caused the most hydrolysis. It is not known what significance to attach to the fact that the lowest "non-protein" value (3.7 per cent.) occurs at pH 9 and that the value increases as the reaction goes in either direction, above or below pH 9. The reaction also has an important effect on the "non-protein" fraction in root wood and spinach.

SALT SERIES

GORTNER, HOFFMAN, and SINCLAIR (**4**) report a very interesting experience with the effect of neutral salts in the extraction of proteins from wheat flour. They found that the anions were very important and can be arranged according to effectiveness in the following order. $F < SO_4 < Cl <$ Tartrate $< Br < I$. Of the cations they used, sodium and potassium were the least effective and calcium the most. Table XI shows the effect of some

neutral salts in 0.1 N concentrations on the extraction of "proteins" from pear leaves. The series from these results would be $SO_4 < I <$ oxalate < tartrate. If it is assumed that potassium chloride would act similarly to sodium chloride then chloride would appear to be less effective than sulphate. In this study sodium was far better than calcium when they were used as chlorides. Calcium salts, even calcium hydroxide in a saturated solution, gave the poorest yields of any salts used. The effects of the ions of neutral salts are quite aside from the effects of hydroxyl ion concentrations, and are quite ineffective in peptizing cytoplasmic nitrogen when compared with the activity of hydroxyl ion.

TABLE XI

INFLUENCE OF SALT SERIES ON EXTRACTION OF NITROGEN FROM LEAF POWDER
ALL VALUES ARE IN TERMS OF PER CENT. OF TOTAL N IN THE SAMPLES

SALT USED, N/10	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN PRECIPITATE "PROTEIN"
	per cent.	*per cent.*	*per cent.*
K Tartrate	24.05	6.05	18.00
K Oxalate	24.7	7.4	17.3
KI	22.0	6.2	15.8
K_2SO_4	20.0	5.9	14.1
NaCl	16.5	7.0	9.5
$CaCl_2$	7.75	6.3	1.45
$Ca(OH)_2$	17.25	9.85	7.40

Tartrate, the best of the anions of neutral salts, when used in the extraction of N from spinach leaves was also quite inactive, when compared with the effectiveness of alkaline solutions. It is shown here that the anions of neutral salts differ from one another in their effectiveness to extract cytoplasmic proteins, but all of them are much inferior to hydroxyl ion in their yield of total extractable N. The ability or inability of various ions to peptize cytoplasmic proteins may through extensive study give an insight into some of the properties of the N constituents of protoplasm.

HYDROLYSIS

So far the best treatment for extraction of nitrogen had been 2N sodium hydroxide, which, in the case of leaf tissue, had extracted about 90 per cent. of the N. The question arose as to whether the extraction could be rendered more complete by means of hydrolysis. Proteolytic enzyme was obtained by the digestion of a fresh pig's stomach. The procedure was to digest 200 gm. of stomach in a liter of water at pH 2, for 24 hours at 45° C. For

digestion of a leaf sample, 15 ml. of this digestion liquid was used to 10 gm. of leaf powder in 250 ml. of water. The reaction and temperature used were the same as for the previous digestion. The hydrolysis was carried out for two periods of time, 2 hours, and 18 hours. Controls were run with pear tissue in water at pH 2 for the same periods of time. In the shorter period 63.5 per cent. of the N was made soluble, while the acid control for the same length of time gave 21 per cent. of the N in solution. In 18 hours the animal enzyme had rendered soluble 68 per cent. of the total N, and the acid control had done nearly as well, making 60.5 per cent. of N soluble. In the sample hydrolyzed for the shorter period it was found that not all of the sample had been thoroughly dispersed. It is quite possible that if it had not been for this mishap, hydrolysis would have proceeded as far in 2 hours as it did in 18 hours.

It is interesting that the acid control for 18 hours yielded nearly as much soluble N as did the animal enzyme. This may indicate activity of the leaf enzymes, for the hydrogen ion concentration in itself was hardly sufficient to accomplish such hydrolysis at the temperature used.

An acid hydrolysis with 18 per cent. hydrochloric acid for 24 hours on a steam bath made 75.6 per cent. of the N soluble, which is slightly better than the results with the animal enzyme.

Practically complete extraction of leaf N was accomplished by first extracting with 70 per cent. hot alcohol containing 1 per cent. acid, which gave 74.9 per cent. soluble N. This was followed by a sodium hydroxide extraction for three days at 40° C. which yielded a further 23.5 per cent., making the total N extracted 98.4 per cent.

Table XII gives an idea of the extractability of the nitrogenous compounds of the different tissues with 0.2 N sodium carbonate solution.

TABLE XII

TOTAL NITROGEN IN Na_2CO_3 EXTRACT OF SAMPLES DRIED AT 50° C.

TISSUE	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN PRECIPITATE "PROTEIN"
	per cent.	*per cent.*	*per cent.*
Leaves	47.50	5.0	42.50
Bark	30.7	8.6	22.1
Wood	34.2	16.4	17.8
Root wood	57.8	56.8	1.0
Spinach	60.9	31.9	29.0

III Distribution of nitrogen in expressed sap

In table XII it is seen that the different tissues of the pear tree, namely, leaves, shoot wood and bark, and root wood, are very different in their N contents and are strikingly unlike in the distribution of their "protein" and "non-protein" fractions. The difference between these tissues in their "non-protein" N would lead one to believe that the cells of these tissues must be functioning with quite different concentrations of the materials that go to make up the "non-protein" fraction. The opportunity to see what might constitute some of these differences was offered by the materials remaining from another investigation, which had required the collection of samples during the winter, and from which sap was expressed by a hydraulic press. Each time that an excess sap was obtained it was saved and preserved at 0° C. with chloroform. The composites thus accumulated during the winter were analyzed for their N fractions. The sap was from bark and wood of the tops and roots of vigorous-growing six-year-old Bartlett pear trees.

The N precipitated by colloidal iron was assumed to be protein N. The filtrate from the iron precipitation was precipitated with phosphotungstic acid. The N thus precipitated when compared with a similar precipitation of a protein free sample subjected to acid hydrolysis with 20 per cent. hydrochloric acid, gave the polypeptide N. The phosphotungstic acid precipitate after the acid hydrolysis gave the diamino acids. Mono-amino acid N was obtained by the usual VAN SLYKE method. Amide N was determined by hydrolyzing the filtrate from phosphotungstic acid precipitates for 2 hours with 4 per cent. HCl and distilling of ammonia after making the solution alkaline with magnesium oxide.

TABLE XIII

DISTRIBUTION OF NITROGEN IN EXPRESSED SAP
ALL VALUES ARE IN TERMS OF PERCENTAGE TOTAL N IN SAP

TISSUES	TOTAL CC. N/10 ACID PER 100 CC. OF SAP	PROTEIN N	POLY-PEPTIDE N	DIAMINO N	MONO-AMINO N	AMIDE N	RESIDUAL N
	cc.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Top bark	49.5	4.25	16.77	2.63	45.0	17.6	13.7
Top wood	154.5	4.30	64.80	6.60	6.2	3.1	15.2
Root bark	42.5	4.95	33.92	1.88	31.6	9.9	17.9
Root wood	154.5	5.25	49.99	3.11	19.7	3.9	18.0

The data in table XIII show that the wood contained three times as much of its N in the sap as in the bark. The amount of protein is relatively small and quite similar in the sap of all the tissues. Nearly half of the N of wood sap is in the form of polypeptides, and the actual amounts in this tissue are very large for more of the wood N is in the expressed sap. The polypeptide N may amount to 25 per cent. of the total N of wood. The quantity of diamino acids is small.

The bark while low in polypeptide and diamino N is very high in monoamino acids; and this may be the highest of all bark N fractions. The bark is also highest in amides, particularly the top bark. The relatively small figures for residual N show that the fractionation has been quite complete. The methods of fractionation are standard and the differences so marked that some positive differences in composition are here indicated.

DEPARTMENT OF HORTICULTURE,
UNIVERSITY OF CALIFORNIA.

LITERATURE CITED

1. CHIBNALL, A. C. The effect of low temperature drying on the distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**: 599–607. 1922.
2. ————, and GROVER, C. E. A study of leaf cell cytoplasm. I. The soluble proteins. Biochem. Jour. **20**: 108–118. 1926.
3. EL SAWY, A. H. Some effects of wounding on respiration and food reserves in the pear tree (*Pyrus communis*). Unpublished dissertation. University of California. 1927.
4. GORTNER, R. A., HOFFMAN, W. F., and SINCLAIR, W. B. Physico-chemical studies on proteins. III. Proteins and the lyotropic series. Colloid Symposium Monograph **5**: 179–198. 1927.
5. NIGHTINGALE, G. T., ROBBINS, W. R., and SCHERMERHORN, L. G. Freezing as a method of preserving plant tissue for the determination of nitrogenous fractions. New Jersey Agr. Exp. Sta. Bull. 448. 1927.
6. OSBORNE, T. B., and WAKEMAN, A. J. The proteins of green leaves. I. Spinach leaves. Jour. Biol. Chem. **42**: 1–26. 1920.
7. THOMAS, ARTHUR W., and FRIEDEN, ALEXANDER. The gelatin-tannin reaction. Jour. Ind. Eng. Chem. **15**: 839–841. 1923.
8. THOMAS, WALTER. Nitrogenous metabolism of *Pyrus malus*, L. Plant Physiol. **2**: 55–66. 1927.

HEMICELLULOSE AS A STORAGE CARBOHYDRATE IN WOODY PLANTS, WITH SPECIAL REFERENCE TO THE APPLE

A. E. MURNEEK

(WITH FIVE FIGURES)

After the removal of sugars and starch by commonly accepted methods from properly prepared woody plant tissues, there usually remains a residue. A large part of this residual cell wall material may be hydrolyzed by dilute mineral acids to monosaccharides or other carbohydrates having the power to reduce copper compounds. Hence the quantity of this "digestible" fraction may be readily estimated. Such higher carbohydrates, other than starch, have been variously designated in literature as "polysaccharides," "acid hydrolyzable material," etc. More often, however, the name "hemicellulose" has been assigned to this group of complex compounds.

In most lignified tissues the relative amount of hemicellulose is very high. Moreover, in certain organs at least, it shows a marked seasonal fluctuation. Hemicellulose, therefore, may be readily available to the plant, possibly through enzymatic action. What is the significance of this cellular aggregation in the physiology of woody plants? Most certainly it can not be ignored in studies of carbohydrate distribution, since more often than not it constitutes the preponderant volume of the more labile forms of these substances.

It is the object of this paper to review briefly our present knowledge of the occurrence, distribution, and chemistry of hemicellulose in some higher plants. It is hoped that thereby further interest may be created in this highly important group of reserve carbohydrates.

Historical retrospect

There is reliable evidence (**20**) that as early as 1862 SACHS observed that upon germination a portion of the cell walls of the date seed disappears, leaving a much thinner permanent layer. Since then it has been shown by many investigators that a storage cellulose of this character is present in various parts of seeds of many plants, but especially in certain kinds of endosperms and cotyledons. After extensive observations and careful chemical studies, REISS, 1889 (*loc. cit.*) came to the conclusion that this labile cellular thickening is not a true cellulose and hence it should be definitely designated by the already suggested names of "amyloid" or "reserve cellulose." Two years later (1891) SCHULZE (**26**) proposed the name "hemicellulose" for all such substances of the plant cell wall, which upon

hydrolysis with weak mineral acids, usually give such sugars as galactose, mannose, arabinose, xylose, etc., thus clearly distinguishing it from the more fibrous cellulose, which is hydrolyzable only by strong acids and yields in most cases but glucose.

That hemicellulose is present in other tissues than those intimately associated with the embryo has been demonstrated by several European investigators but especially by DU SABLON (**5**) and SCHELLENBERG (**23**). Using as the material a number of typical woody plants, the former has shown by chemical analysis that hemicellulose accumulates in the fall, reaching a maximum in October in various vegetative organs, but particularly in twigs and roots. It is mobilized and reabsorbed during the following spring and usually shows a minimum concentration in summer (May–June). Microscopic observation of willow twigs collected in February showed clearly an incrustation interior to the cell wall proper. This thickening disappeared in summer and could be removed with hydrochloric acid from twigs collected in midwinter.

SCHELLENBERG likewise observed that hemicellulose is laid down and utilized as a reserve food in different kinds of tissues of lignified plants, but especially in sieve tubes, wood fibers, and cortex parenchyma. It is removed in the spring from parenchyma cells throughout the plant, from the tips of branches to ends of roots. He points out the fact, moreover, that cells immediately below an inflorescence show conspicuously the disappearance of wall thickenings during flowering. Another interesting observation made by SCHELLENBERG (**22**) is that in various organs of *Molinia coerulea*, when cell wall thickenings appear after leaf fall (Oct.–Nov.), starch disappears, and when they are redissolved in the spring starch is present once more, thus suggesting a possible connection between starch and hemicellulose. In all instances hemicellulose of this nature could be removed by hydrolysis with hydrochloric and sulphuric acids (3–5 per cent.).

Since then many investigators have noted the hemicellulose layer in cells of various plants and have made attempts to study its chemical nature. The technical horticulturists in particular have determined the presence and the relative amounts of these substances in a number of deciduous trees, especially the apple.

Hemicellulose in apple tissues

Although hemicellulose has been included in chemical analysis of apple wood (**12, 9, 7, 13, 2**) it has not been recognized as such but has been commonly referred to by the already mentioned terms "polysaccharides," "acid hydrolyzable polysaccharides," etc. This terminology, if justifiable at all, should be used only in cases where starch and other hydrolyzable material has been included in this fraction. Recently, however, the name

"hemicellulose" has come to the forefront. Moreover, it has been recognized that the various sugars and starches are not the only carbohydrates that enter into the metabolism or may serve as storage products, but that hemicellulose should be considered as an important reserve substance in

TABLE I

STARCH (AS DEXTROSE) IN PERCENTAGES OF DRY WEIGHT IN TWO VARIETIES OF BEARING APPLE SPURS, 1925

VARIETAL TISSUES	APRIL 15	MAY 1	MAY 15	JUNE 15	JULY 15	AUGUST 15
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Jonathan						
Leaves	0.40	2.45	3.30	2.40	1.35	.....
New growth	2.15	2.25	3.75	1.15	3.75	.....
Flowers-fruit	1.00	2.40	3.60	9.15	14.30	11.20
Paynes Late Keeper						
Leaves	0.75	2.10	4.35	2.80	1.90	.....
New growth	2.00	2.10	4.25	0.40	4.60	.....
Flowers-fruit	0.25	1.75	7.45	10.00	11.95	17.70

TABLE II

HEMICELLULOSE (AS DEXTROSE) IN PERCENTAGES OF DRY WEIGHT IN TWO VARIETIES OF BEARING APPLE SPURS, 1925

VARIETAL TISSUES	APRIL 15	MAY 1	MAY 15	JUNE 15	JULY 15	AUGUST 15
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Jonathan						
Leaves	14.50	16.30	13.70	17.80	15.95	.....
New growth	18.20	17.90	16.60	20.40	21.65	.....
Flowers-fruit	16.80	13.60	15.20	22.05	18.30	9.55
Paynes Late Keeper						
Leaves	15.55	17.65	14.35	17.25	15.70	.....
New growth	18.90	17.00	18.20	18.75	21.65	.....
Flowers-fruit	15.25	14.55	15.70	21.25	22.55	13.95

apple tissues. TOTTINGHAM, *et al.* (**29**), for instance, have shown that when sugars and starch are removed from apple wood the residue, upon hydrolysis, will produce reducing substances that in most instances represent 2 to 5 times the quantity of both sugars and starch combined. A detailed examination of the products of hydrolysis of this hemicellulose showed that it consists primarily of xylose and glucose with a small amount of galactose. ROBERTS (**21**) too is of the opinion that in the apple the carbohydrate reserve, in the form of cell wall thickenings, constitutes a greater quantity

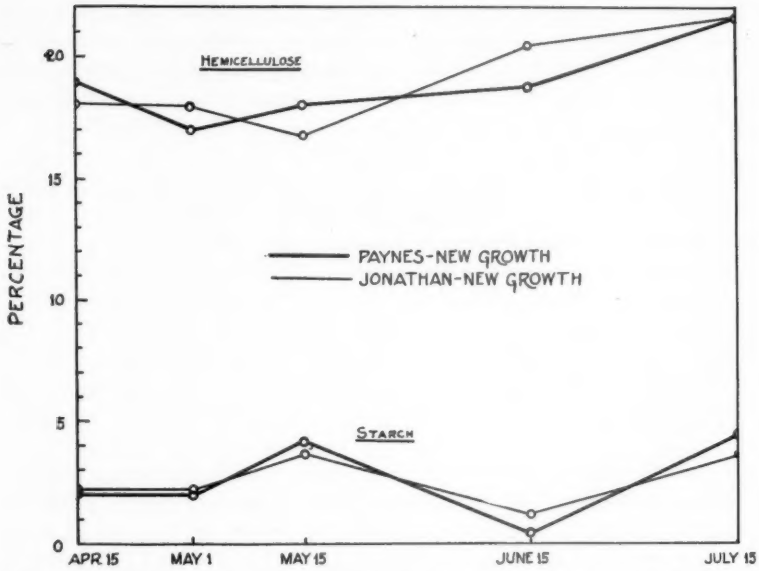


FIG. 1. Hemicellulose and starch concentration, in percentages of dry weight, in new growth of two varieties of bearing apple spurs, 1925.

than that of starch, and that it is laid down primarily in the fall and disappears when growth is resumed. And HARVEY (**8**) has shown that of the total polysaccharides in apple shoots, by far the largest fraction is made up of pentoses, thus indicating that hemicelluloses are primarily pentosans, as was suggested by SPOEHR (**27**).

In a recent investigation on the nitrogen and carbohydrate distribution in organs of two varieties of apple spurs the writer (**16**) has noted once more the conspicuously large amounts of hemicellulose in certain tissues. While during the summer the starch content in the new growth of the fruiting spur may constitute 1 to 4 per cent. of the total dry matter (table I), hemicellulose represents 17 to 22 per cent. (table II and fig. 1). Similar proportions of these two groups of substances were found also in leaves, flowers, and fruits (fig. 2). Hemicellulose appears to be formed in great quantity in the developing fruits, which show a consistent increase to a maximum concentration by midsummer, followed by a rapid decrease later on (fig. 2). The data suggest that hemicellulose is laid down in the flesh or cortex of the fruit as a reserve food in the form of accretions to cell walls which during the later part of the development are hydrolyzed through enzymatic action (see also Amer. Journ. Bot. **10**: 310–324. 1923). Thus in

TABLE III

HEMICELLULOSE (AS DEXTROSE) IN PERCENTAGES OF DRY WEIGHT IN TWO VARIETIES OF BEARING APPLE SPURS, 1926

VARIETAL TISSUE	APRIL 26	MAY 1	MAY 6	MAY 11	MAY 17	MAY 22	MAY 27	JUNE 1	JUNE 7	JUNE 12
Jonathan										
Leaves	26.70	27.6	25.2	31.1	28.9	35.5	34.7	30.7	28.10	30.00
New growth	39.6	39.1	21.5	49.7	32.7	29.7	27.1	36.75	28.10	38.45
Flowers-fruit	37.2	29.05	19.1	32.15	32.2	27.5	26.3	26.3	25.80	31.45
King David										
Leaves	27.05	29.3	27.5	33.2	27.6	31.9	29.1	31.65	29.55	30.45
New growth	35.8	35.4	19.8	49.7	35.4	37.93	36.85	36.8	33.20	37.2
Flowers-fruit	28.3	29.35	21.3	29.4	31.2	25.95	27.45	28.3	27.55	33.5

addition to starch, evidently it is a source of increasing sugar content for the maturing fruit.[1]

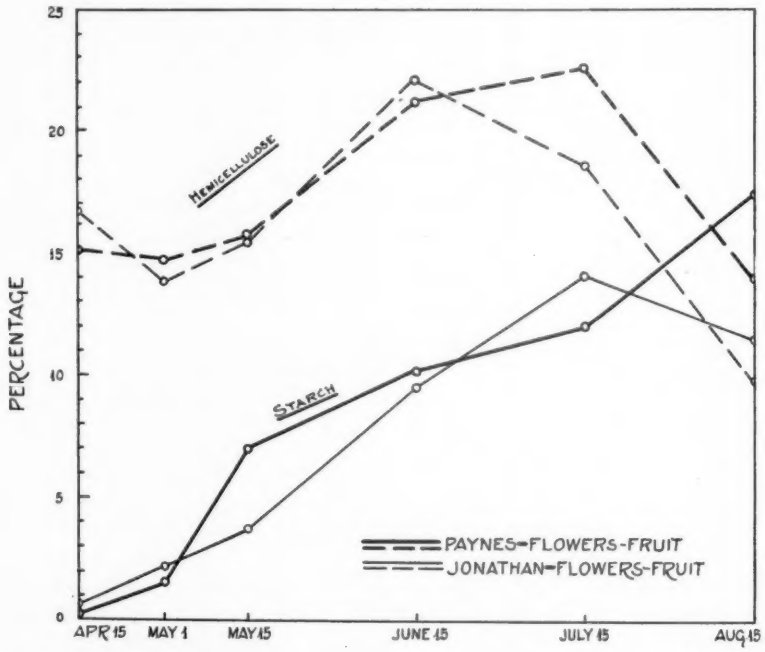


FIG. 2. Hemicellulose and starch concentration, in percentages of dry weight, in reproductive organs (flowers-fruit) of two varieties of bearing apple spurs, 1925.

When comparable material was analyzed at short 5- to 6-day intervals, other interesting features regarding the behavior of hemicellulose in various organs of the fruiting apple spur were revealed. A very rapid reduction in hemicellulose content seems to have taken place in the new growth and the flowers at the time of anthesis—May 3–9 (table III and fig. 3). And since at the exact time when the concentration of hemicellulose was at a minimum the quantity of total sugars is correspondingly high (table IV and fig. 4), it is more than likely that much of the hemicellulose had been converted into sugars during this period and again recondensed after petal and flower abscission. The starch content, not undergoing an equally rapid fluctuation during this time of intense development (fig. 5), suggests that starch after all is not the most important and most available storage form

[1] It is conceivable that various "pectin" substances may function in the same capacity in the apple fruit and that they may have been included in this determination.

TABLE IV

TOTAL SUGARS (AS DEXTROSE) IN PERCENTAGES OF DRY WEIGHT IN TWO VARIETIES OF BEARING APPLE SPURS, 1926

VARIETAL TISSUE	APRIL 26	MAY 1	MAY 6	MAY 11	MAY 17	MAY 22	MAY 27	JUNE 1	JUNE 7	JUNE 12
Jonathan										
Leaves	4.20	6.30	4.85	3.90	3.00	2.90	4.65	4.50	3.90	5.15
New growth	4.80	3.50	2.33	2.00	1.30	2.60	2.40	2.90	2.80	2.15
Flowers-fruit	2.60	6.20	11.95	2.75	2.95	2.95	4.60	4.80	7.45	7.50
King David										
Leaves	3.45	5.15	7.40	6.10	6.10	4.50	5.35	5.65	4.60	4.90
New growth	.....	4.20	3.45	2.93	1.35	2.93	2.25	2.90	2.35	2.45
Flowers-fruit	2.40	3.30	12.20	4.50	3.05	2.75	4.80	6.15	.....	9.30

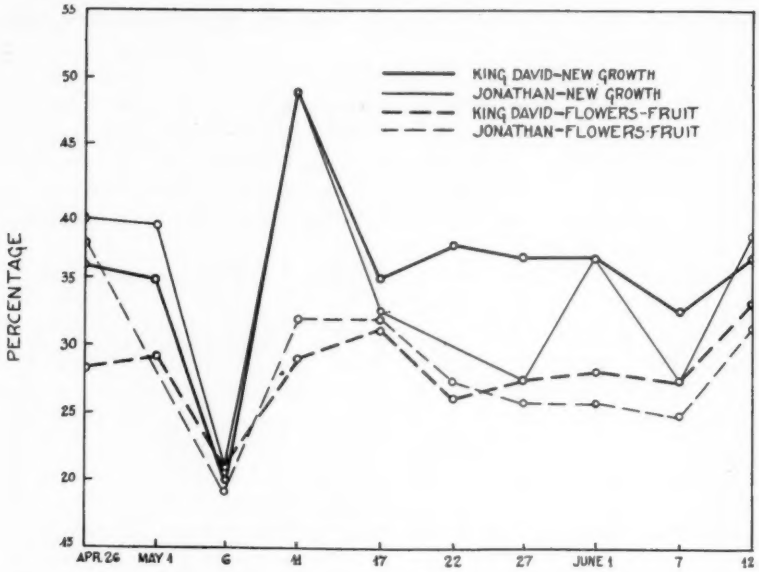


FIG. 3. Graphs showing changes in hemicellulose content during flowering and fruit setting in new growth and reproductive organs (flowers-fruit) of two varieties of bearing apple spurs, 1926. Percentages on dry weight basis.

of carbohydrates in the apple as has been postulated by some investigators. In fact, the percentage of starch in the new growth of the spur actually increased during flowering (May 6, fig. 5)—the period of greatest reduction in the hemicellulose content. Hence there is a possibility that, concomitant with the rapid increase in sugar content, starch may have been synthesized from the products of hydrolysis of hemicellulose. This seems to point to a starch-hemicellulose equilibrium and thereby support is given to SCHELLENBERG'S observations (**22**).

These studies emphasize once more the importance of hemicellulose as a reserve carbohydrate in woody plants. Hence this group of substances most certainly merits further detailed study with special emphasis on the chemical constitution and metabolic rôle of each constituent.

Chemistry of hemicelluloses

Although one is forced to agree with SPOEHR (*loc. cit.*) that though much literature has accumulated on the subject of the colloidal polysaccharides (including hemicelluloses), the "opinions are widely divergent and the subject in general is a most uncoordinated one," still, lately a number

of contributions have appeared on the chemistry of hemicelluloses which seem to merit our attention.

In the make up of plant cell walls three major substances commonly accompany cellulose. They are: lignin, hemicellulose, and pectin. None of them, however, as yet has been isolated in chemically pure form. From all appearance they seem to be closely related to each other, this being particularly true of pectin and hemicellulose. Consequently any progress made in the chemistry of one of these groups of substances may throw light upon the others.

In addition to cellulose, woody tissues usually contain lignin and hemicellulose while non-lignified portions of plants show only small amounts of hemicellulose, but relatively large aggregations of pectin. During lignification pectin seems to disappear and is replaced by hemicellulose and lignin. CANDLIN and SCHRYVER (**3**) even go so far as to advance the idea that hemicellulose in lignified cells is derived from pectin and that there is a possibility, though remote as yet, that certain products obtained from pectin may be related to lignin. EHRLICH (**6**) likewise says that hemicellulose is closely related to pectin and that lignin is probably formed from pectin by oxidation processes. Thus it is at least suggestive that these three

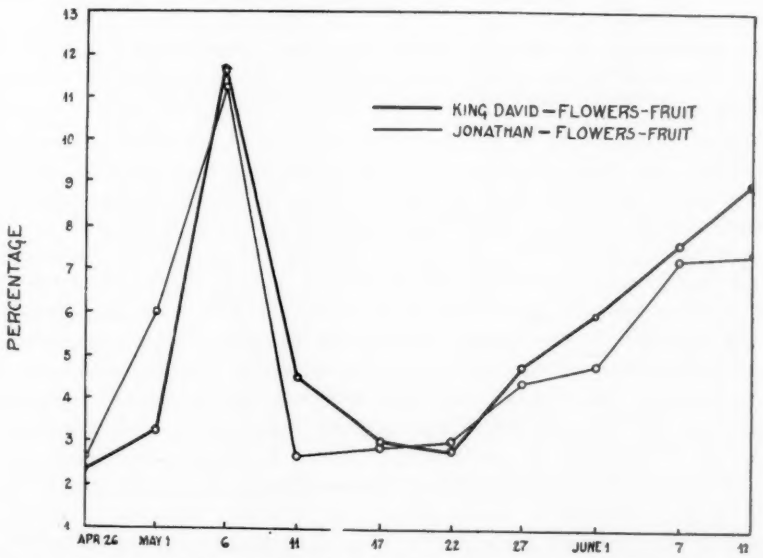


FIG. 4. Graphs showing changes in total sugar content during flowering and fruit setting in reproductive organs (flowers-fruit) of two varieties of bearing apple spurs, 1926. Percentages on dry weight basis.

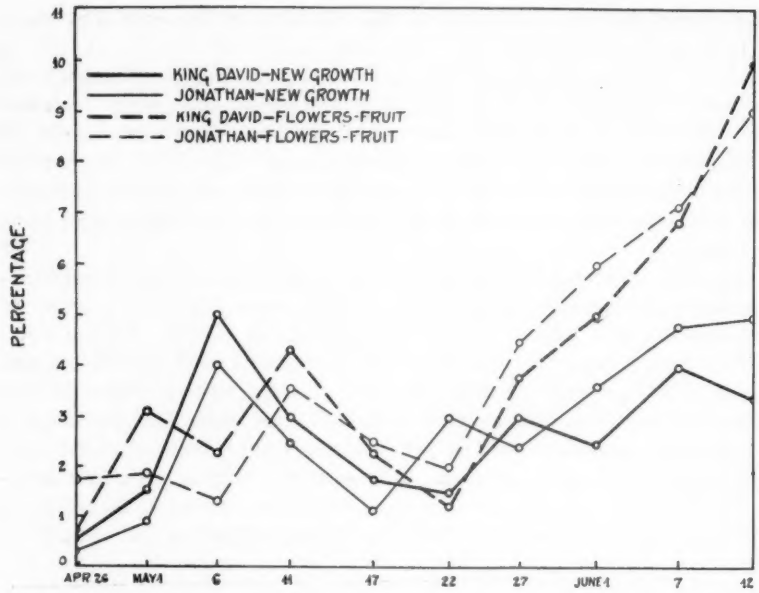


FIG. 5. Graphs showing changes in starch content during flowering and fruit setting in new growth and reproductive organs (flowers-fruit) of two varieties of bearing apple spurs, 1926. Percentages on dry weight basis.

interesting compounds of the plant cell wall may be generically connected. Furthermore, recent studies by SCHRYVER and coworkers (**4, 25**) and by LING and NANJI (**14**) seem to indicate that a substance of the nature of hemicellulose ("amylo-hemicellulose") is present in various starches (sago, maize, wheat, rice, tapioca, potato). Unlike the main constituents of starch, this hemicellulose is not digested by diastase. When hydrolyzed by acids it yields largely glucose. Some of the authors (**14**) believe that "amylo-hemicellulose" is present not only in starch but also as a principal constituent of cell walls of many plant organs, especially during early stages of development. As it colors blue with iodine it may be taken for starch. THAYSEN and BUNKER (**28**) think that hemicellulose probably is more common in plant tissues than cellulose, but is less thoroughly investigated.

A number of reagents have been used for the extraction of hemicellulose but a dilute solution of caustic soda (2–4 per cent.) (**18**) and hot water, with or without autoclaving (**10**), seem to have been preferred. While for the extraction of pectin a weak solution (0.5 per cent.) of ammonium oxalate (*loc. cit.* **3**) is usually employed, although hot and cold water have also been used extensively. Therefore, when literature on this phase of the

subject is reviewed one can not help gaining the impression that, with some plant material at least, a water extract may not represent pure hemicellulose or pectin but a mixture of the two, and that a solution of alkali (4 per cent.) most likely decomposes hemicellulose. Evidently a proper medium for the separation of hemicellulose from other cell wall constituents is yet to be found.

When a detailed chemical investigation is undertaken, the extracted hemicellulose, with or without further purification, is usually subjected to an acid hydrolysis. The end products of such a treatment have been popularly considered as consisting of a mixture of several monosaccharides —hexoses and pentoses. The following sugars have been often identified as the products of hydrolysis of various hemicelluloses: glucose, galactose, mannose, arabinose, xylose and others. While some workers are of the opinion that mannans and galactans are the most common forms of reserve celluloses, most hemicelluloses evidently are made up largely of pentoses and hence may be considered as pentosans. PRINGSHEIM (**19**), however, has isolated from certain hemicelluloses a heretofore unknown trisaccharide and a dimannan.

Lately quite convincing proof has been secured that substances of the type of galacturonic and glycuronic acids and their polymeric anhydrides, the poly-galacturonic and -glycuronic acids appear to be the main building stones from which plant cell membranes (hemicelluloses), and pectins as well, are constructed (**6, 18**). This particular group of polysaccharides has the characteristics of both carbohydrates and acids. And since these "uronic" acids either make up the nucleus (**15, 1**) or constitute a large fraction of the pectin compounds (**24**), this may be considered as another link in the evidence of the close relationship between pectins and hemicelluloses. Thus both of these cell wall constituents may be called "polyuronides."

Pectins probably contain more uronic acids than hemicelluloses, but lignin contains none (**3**). It may be of further interest to note that according to CANDLIN and SCHRYVER, pectin, when treated with weak alkali yields among other products hemicelluloses, which still contain uronic acids and resemble in some respects hemicelluloses isolated directly from timber. It is quite evident then that a rather interesting field of investigation has been opened by recent studies of the various complex carbohydrates which are not merely aggregates of the plant cell wall but also serve as reserve substances.

In spite of the somewhat involved chemistry and the complex "uronic" acids in particular, the distribution and rôle of hemicellulose should be of special interest to plant physiologists. In woody plants these

substances are usually present in very large amounts and frequently serve as reserve carbohydrates. Moreover, the probable relationship between starch, pectin, hemicellulose and lignin is very interesting indeed. As pentosans, hemicelluloses seem to be very effective in making plants resistant to drought and to cold. This special field of investigation has been only tentatively explored. Finally the hemicelluloses seem to be of consequence in relation to the carbohydrate-nitrogen ratio in plants. Frequently the acid hydrolyzable polysaccharides (hemicelluloses, or hemicelluloses and starch) constitute the preponderant amount of all the available and extractable carbohydrates and consequently may successfully represent them in the ratio (**17**). Moreover, HICKS (**11**) has shown rather clearly that the ratio of total elemental carbon to total elemental nitrogen, the true C/N ratio, may be just as significant as that of carbohydrates to nitrogen. One should remember that in many instances the hemicellulose represents a very large part of the carbon and the carbohydrates.

UNIVERSITY OF MISSOURI,
COLUMBIA, MISSOURI

LITERATURE CITED[2]

1. AHMANN, C. F. and HOOKER, H. D. The estimation of pectin and a study of the constitution of pectin. Missouri Agr. Exp. Sta. Res. Bull. **77**. 1925.
2. AUCHTER, E. C., *et al.* The effect of shade on the growth, fruit bud formation, and chemical composition of apple trees. Proc. Amer. Soc. Hort. Sci. 1926: 368–382.
3. CANDLIN, E. J. and SCHRYVER, S. B. Investigations of the cell-wall substances of plants, with special reference to the chemical changes taking place during lignification. Proc. Roy. Soc. (London) B. **103**: 365–376. 1928.
4. CLAYSON, D. H. F. and SCHRYVER, S. B. The hemicelluloses. I. The hemicellulose of wheat flour. Biochem. Jour. **17**: 493–496. 1923.
5. DU SABLON, LECLERC. Recherches physiologiques sur les matières de réserves des arbres. Rev. Gen. Bot. **16**: 341–368, 386–401. 1904.
6. EHRLICH, F. Neue Untersuchungen über Pektinstoffe. Zeitschr. Ang. Chem. **40**: 1305–1313. 1927.
7. HARLEY, C. P. Normal variations in the chemical composition of fruit spurs and the relation of composition to fruit bud formation. Proc. Amer. Soc. Hort. Sci. 1925: 134–146.

[2] Acknowledgment is due to Prof. W. E. PYKE, of the Department of Chemistry, Colorado Agricultural College, for suggesting some of the references.

8. HARVEY, E. M. A study of growth in summer shoots of the apple with special consideration of the rôle of carbohydrates and nitrogen. Oregon Agr. Exp. Sta. Bull. 200. 1923.
9. ————, and MURNEEK, A. E. The relation of carbohydrates and nitrogen to the behavior of apple spurs. Oregon Agr. Exp. Sta. Bull. 176. 1921.
10. HENDERSON, S. T. The pectin and hemicelluloses of the flax plant. Jour. Chem. Soc. (London) **130**: 2117–2125. 1928.
11. HICKS, P. A. The carbon-nitrogen ratio in the wheat plant. New Phyt. **27**: 1–46. 1928. *Ibid.* **27**: 108–116. 1928.
12. HOOKER, H. D. Seasonal changes in the chemical composition of apple spurs. Missouri Agr. Exp. Sta. Res. Bull. 40. 1920.
13. KRAYBILL, H. R., *et al.* Some chemical constituents of fruit spurs associated with blossom bud formation in the Baldwin apple. New Hampshire Agr. Exp. Sta. Tech. Bull. 29. 1925.
14. LING, A. R. and NANJI, D. R. Studies on starch. Part IV. The nature of the amylo-hemicellulose constituent of certain starches. Jour. Chem. Soc. (London) **127**: 652–656. 1925.
15. MCKINNIS, R. B. An investigation of the hypothetical combined pentose and the so-called free pentose with inferences on the composition of pectin. Jour. Amer. Chem. Soc. **50**: 1911–1915. 1928.
16. MURNEEK, A. E. Nitrogen and carbohydrate distribution in organs of bearing apple spurs. Missouri Agr. Exp. Sta. Res. Bull. 119. 1928.
17. ————. Effects of pruning on the carbonhydrate-nitrogen ratio in the tomato. Proc. Amer. Soc. Hort. Sci. 1927: 180–184.
18. O'DWYER, M. H. The hemicelluloses. III. The hemicelluloses of American white oak. Biochem. Jour. **17**: 501–509. 1923. *Ibid.* **20**: 656–664. 1926; **22**: 381–390. 1928.
19. PRINGSHEIM, H. Über den fermentativen Abbau der Hemicellulosen. I. Ein Trisaccharid als Zwischenproduct der Hydrolyse eines Mannans. Zeitschr. Physiol. Chem. **80**: 376–382. 1912. *Ibid.* **123**: 205–212. 1922.
20. REISS, R. Über die Natur der Reservecellulose und über ihre Auflösungsweise bei der Keimung der Samen. Landw. Jahrb. **18**: 711–765. 1889.
21. ROBERTS, R. H. Apple physiology. Wisconsin Agr. Exp. Sta. Res. Bull. 68. 1926.
22. SCHELLENBERG, H. C. Über die Bestockungsverhältnisse von *Molinia coerulea* Mönch. Ber. Schweiz. bot. Ges. 1897.
23. ————. Über Hemicellulose als Reservestoffe bei unseren Waldbäumen. Ber. deutsch. bot. Ges. **23**: 36–48. 1905.

24. SCHRYVER, S. B. and NORRIS, F. W. The pectic substances of plants. III. The nature of pectinogen and its relation to pectic acid. Biochem. Jour. **19**: 676–693. 1925.

25. ———— and THOMAS, E. M. The hemicelluloses. II. The hemicellulose contents of starches. Biochem. Jour. **17**: 497–500. 1923.

26. SCHULZE, E. Zur Kenntniss der chemischen Zusammensetzung der pflanzlichen Zellmembranen. Ber. deutsch. chem. Ges. **24**: 2277–2287. 1891. *Idem.* Zeitschr. Physiol. Chem. **16**: 387–438. 1892.

27. SPOEHR, H. A. The carbohydrate economy of cacti. Carnegie Inst. Washington. Publ. no. 287. 1919.

28. THAYSEN, A. C., and BUNKER, H. J. The microbiology of cellulose, hemicellulose, pectin, and gums. Oxford Univ. Press. 1927.

29. TOTTINGHAM, W. E., *et al.* Hemicellulose in apple wood. Jour. Biol. Chem. **45**: 407–414. 1921.

IDENTIFICATION OF CERTAIN SPECIES OF CITRUS BY COLORIMETRIC TESTS[1]

F. F. Halma and A. R. C. Haas

Citrus varieties are propagated commercially by budding. In California four species are used for rootstocks and in the order of their importance, they are as follows: Sweet orange (*Citrus sinensis* Osbeck), sour orange (*Citrus aurantium* L.), grapefruit (*Citrus maxima* Merrill), and rough lemon (*Citrus limonia*). *Poncirus trifoliata* Raf., used formerly to a limited extent, is being discarded.

At the present time considerable thought is being given to the selection of stocks which are congenial with the scion variety. Owing to the fact that formerly very little attention was paid to this phase of citriculture, we now find a considerable mixture of stocks in older plantings. Such old orchards would offer excellent opportunity to study the interrelation of stock and scion were it possible to identify definitely the stock in question. Up to the present time there have been no generally applicable methods by which this could be done. Occasionally one finds a sprout growing from below the bud union, or from a cut root, from which it is possible to make a diagnosis. The type of bud union sometimes suggests the stock variety, but it is always difficult to distinguish between sweet orange and sour orange stocks, especially with old trees.

For the past two years considerable effort has been made to devise a method which would at least make it possible to separate sweet orange from sour orange stocks with certainty. It is obvious that any adequate method must be accurate and fairly rapid, and must require very little material. It may be mentioned that a separation on the basis of anatomical differences was found to be unsatisfactory. Water extracts of powdered bark when tested with different pH indicators (within, as well as outside of the range of citrus-bark acidity), gave inconsistent results.

Recently Hendricksen (**2**) published a method which is based on the fact that all Citrus roots contain varying amounts of glucosides and that these glucosides contain phenols. By treating an aqueous extract of ground fresh roots with ferric chloride, he observed different shades of color with the sour orange, "wild" grapefruit, and Rough lemon. Sweet orange gave inconsistent results. As a matter of fact Hendricksen considers this method reliable only for Rough lemon, and hence it is of very little value for California conditions. There is the additional disadvantage of having to work with fresh material.

[1] Paper no. 206, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

This paper reports a series of successful tests which were carried on with powdered bark. Of the numerous reagents used, some of which will be mentioned later, the ALMÉN test (**1**) for carbolic or salicylic acid gave the most consistent results. This reagent is prepared as follows: Saturate fuming nitric acid with mercury and dilute the solution with double its volume of water. The degree of sensitiveness is given as 1:400,000. This reagent is practically the same as Millon's reagent for albumins and phenols (**1**).

The procedure which has given excellent results is as follows: Enough bark is peeled from the trunk below the bud union to make at least one gram when dried at about 70° C. Adhering soil or foreign matter, of course, must be removed from the bark before taking the sample. The dried bark is ground fine enough in a mortar to pass through a 40-mesh sieve. One gram of this powder is placed in a beaker and 20 cc. of distilled water are added. In a few minutes the solution is ready for filtration. The contents of the beaker are transferred to filter paper and the residue is washed with two successive 20-cc. portions of distilled water. The one gram of powder is therefore leached with 60 cc. (altogether) of water.

Ten cubic centimeters of the filtrate are measured into a test tube and 8 drops of the ALMÉN reagent are added. A heavy yellowish precipitate forms immediately. The contents of the tube are then boiled a few minutes in a water-bath or in an open flame. Within a short time the heavy precipitate assumes varying shades of color and the solution itself becomes colored. Even at this stage it is often possible to distinguish sweet orange by its deep pink color, but it is best to let the solution cool and the precipitate settle before a critical examination is made. Frequent shaking seems to accentuate the color of the solution upon settling.

With sweet orange the solution assumes a characteristic pink color which persists for several weeks. The grapefruit solution shows a much lighter pink, and the color fades much sooner than that developed by sweet orange. Sour orange usually develops a light pink color and quite often it is difficult to distinguish it from that given by grapefruit. It was found, however, that this difficulty can largely be overcome by frequently shaking the solutions and allowing them to stand several hours. The Rough lemon gives the lightest color of all and with a little experience no special difficulties are encountered in its determination.

A modification of this method which shows greater color differences in a much shorter period, is as follows: Ten cubic centimeters of the filtrate are made alkaline with a few drops of potassium hydroxide prior to the addition of 8 drops of a saturated solution of copper sulphate. This results in the formation of a heavy precipitate. Eight drops of the mercury reagent are then added and the contents of the tube are boiled. The color of the sweet orange solution becomes pink as in the other method but the sour

orange solution assumes a brown color which can be at once distinguished from that of sweet orange. The color of the grapefruit solution becomes a light pink and that of the Rough lemon almost colorless. The sweet orange solutions show a very narrow range of pink while the sour orange and grapefruit solutions fluctuate in some cases over a comparatively wide range within their color group.

If added confidence is needed the remaining portion of the liquid may be used in the following supplementary tests. These methods will be found helpful, although they are not as reliable as those described above; they separate the species only roughly in some cases, but at other times quite definitely. Only 5 cc. of solution are needed for each of the additional tests.

Ammonium molybdate is the reagent ordinarily used for phosphorus determinations and is prepared as follows: Dissolve 100 grams of molybdic acid in a mixture of 144 cc. of ammonium hydroxide and 271 cc. of water. Pour slowly into a cool mixture of 489 cc. of nitric acid and 1148 cc. of water. Ten drops of this reagent added to the liquid and boiled, will give rather consistently a deep blue color with Rough lemon and a much lighter blue or bluish gray with grapefruit. Sweet and sour orange give varying shades of green.

The addition of 10 drops of a saturated solution of copper sulphate roughly separates sweet and sour orange as a single group from grapefruit and Rough lemon as another group. The color differences can be somewhat intensified by the addition of 2 drops of aniline violet gentian (0.01 gram dissolved in 75 cc. of water).

Two drops of titanous chlorid (20 per cent. solution) gives a very light pink color with Rough lemon, a warm gray with grapefruit, and a greenish color with both sweet and sour orange.

Two drops of a saturated solution of ferric chlorid often give a brownish fluorescent turbidity with sweet orange, a dark clear brown color with sour orange, and a clear straw color with Rough lemon and grapefruit (Rough lemon solution generally gives a lighter color than grapefruit).

Many other reagents were tested some of which gave promising results, but the consideration of which would add very little to the previous discussion. The diversity of reagents used precludes any definite statement as regards the nature of the principle involved. It may be mentioned that twelve different phenol reagents gave negative results. Even the mercury reagent gave results somewhat different from the characteristic reactions obtained with phenol or protein.

In a preliminary trial of these methods of identification, bark samples from trees of definitely known variety were examined. More than 100 samples each of sweet orange and sour orange bark, more than 50 of grapefruit

bark, and about 20 of Rough lemon bark were tested without a single discrepancy.

A considerable number of unknown bark samples representing the four kinds of rootstocks have also been successfully identified. A set of 37 samples of bark (11 each of sour, sweet, and grapefruit, and 4 of Rough lemon) was obtained from the citrus breeding orchard of the Citrus Experiment Station and a set of 100 samples representing both sweet and sour orange rootstocks. In each case we determined numbered samples, to whose identity we had no clue aside from the chemical tests, and the list of determinations was given to a cooperator who had retained the only key to the sample numbers. All samples were correctly determined. The odds against the accidental occurrence of this result are obviously enormously large.

The results of these tests are not influenced by the age of the tree, by the time of the year at which the samples were taken, nor by the scion variety; in fact, the color obtained within a certain group is the same regardless of whether it serves as scion or as stock.

With the exception mentioned below, the results with the mercury test have been uniform within the commonly accepted limits of the species here considered. The sweet orange barks tested include, in addition to rootstocks (probably representing the "Mission seedling" type) the varieties Ruby, Valencia, and Washington Navel, while the known grapefruit varieties include Marsh, Duncan, Triumph, and the decidedly different Imperial. The sour orange barks tested undoubtedly represent a mixed lot of unknown origin. On the other hand, the standard California lemon varieties (Lisbon and Eureka) give a characteristic rusty red color which is quite different from that obtained with Rough lemon, although all these lemons are commonly included in *Citrus limonia*. These results suggest the possibility that these colorimetric differences may be useful in citrus classification.

CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA.

LITERATURE CITED

1. COHN, A. I. Tests and reagents. John Wiley and Sons, New York. 1909.
2. HENDRICKSEN, H. C. Identification of citrus budding stock: Porto Rican method for testing root system. Florida Grower **36**: 14–15. 1928.

THE EFFECT OF POTASSIUM, NITROGEN AND PHOSPHORUS FERTILIZING UPON THE CHLOROPLAST PIGMENTS, UPON THE MINERAL CONTENT OF THE LEAVES, AND UPON PRODUCTION IN CROP PLANTS[1]

F. M. SCHERTZ

Introduction

In a former paper (**8**) by the writer, the effect of nitrogen on the chloroplast pigments has been especially indicated. The effect of nitrogen upon the growth of plants is perhaps greater than that of any of the other elements.

VILLE (**14**) was perhaps the first to make any quantitative experiments on the effect of the various fertilizers upon the pigment content of plants. He has shown that nitrogen plays a rôle more important than that of any of the other elements tested. If the dose of nitrogen was increased or diminished the color increased or diminished also.

URBAN (**13**) found evidence of a correlation between the color of the leaves of beets and the nitrogen content, the darker leaves containing the most nitrogen and the lighter the least. Also, he apparently found a relation between the highest potassium content of the leaves and the greatest sugar content of the roots. In beets the dark colored leaves contained more potassium and less sodium than did the light colored leaves. In the leaves of ripening beets the potassium rapidly increases while the sodium decreases.

A great difference in the chlorophyll content of alpine and of lowland plants was observed by HENRICI (**2**). She represented the amount of chlorophyll present in alpine plants as 100 per cent. and found that lowland plants contained 230 per cent. and ravine plants 350 per cent. She makes no reference to the soil in which these grew but it is highly probable that the soil was a very important factor in the amount of chlorophyll which was found in the plants studied.

Perhaps the first real experiments on the effect of chemical fertilizers upon chlorophyll were conducted by WLODEK (**16**) in 1920. Green leaves of potato plants and sugar beets were studied. These were grown in soils to which various fertilizers were added: (1) without fertilizer; (2) with phosphorus, potash and nitrogen; (3) with phosphorus and nitrogen but no potash; (4) with phosphorus, nitrogen and magnesium but no potash; (5) with potash and nitrogen but no phosphorus; and (6) with potash and phosphorus but no nitrogen. After a certain period of development of the

[1] Soil Fertility Investigations, United States Department of Agriculture, Washington, D. C.

plants it was concluded that the relation of the chlorophyll pigments varies during the course of 24 hours; β increases during the day and α during the night. The lack of potash resulted in an absolute and relative diminution of β and an increase in α as well as a reduction in the daily variation of the two components. Lack of phosphorus also reduces the daily variation of the chlorophyll components and narrows the absorption bands. Nitrogen tends to reduce α and to augment β. The action of calcium and magnesium were not definitely established.

In another paper WLODEK (**17**) has reported that with a lack of potash the chlorophyll coefficient diminished at a certain phase of development of the plants. This is shown by an increase in the width of the absorption band of chlorophyll α and a decrease in that of chlorophyll β, as compared with plants grown under normal conditions. A lack of nitrogen in the soil increases the chlorophyll coefficient. A relation seems to exist between the width of the first absorption band and the nitrogen content in fresh bean leaves, and in the straw and harvest of oats and barley. The nitrogen content increases with the width of the absorption band. When potash is lacking the chlorophyll coefficient does not change under the influence of light and darkness. In leaves which show an abnormal chlorophyll coefficient the amount of vegetative material which is produced is less than in those which possess the normal chlorophyll coefficient.

In studying the bush characteristics of potato plants, SCHAEFER (**7**) has placed the leaves in four groups as to color. The classes were, bright green, green, green to dark green, and dark green. He thought that possibly nitrogen affected the color.

WEISSMANN (**15**) noticed that potash influenced the development of plants and their morphological and anatomical structures. He found also that deficient potash affects the coloring of the leaf, stem and the grain. Deficient phosphorus and nitrogen more strikingly affect the early stages of growth than does a deficiency in potash.

In making chemical analyses of leaves, CHIBNALL (**1**) learned that there was a nitrogen withdrawal from the leaves at night. This fact may aid us in understanding the nitrogen metabolism of the leaf.

Studies by MAIWALD (**3**) lead to the conclusion that different applications of potassium produced a great difference in the color of the potato leaves of the same species. The leaves varied in color from a dark green to a yellow green, the yellow green ones resulting when much potash was applied. When based on green weight, deviations of the chlorophyll content were found to be 70 per cent. above and below normal. The amount of the chlorophyll present was the sole cause of the striking color difference in the leaves. By comparing the assimilating power of the plant with the

chlorophyll content, the author concludes that the abnormally low chlorophyll content is the beginning of chlorosis.

Potassium sulphate was found to reduce the chlorophyll content of sound green potato leaves, while kainit and potassium chloride were found by REMY and LIESEGANG (**5**) to greatly reduce the amount of chlorophyll present. Healthy leaves of pronounced potash hungry plants always contained more chlorophyll per unit weight than potash satisfied plants; the same is also true of sugar beets. Leaves of potatoes which had been treated with large amounts of potash salts contained less chlorophyll than did leaves from potatoes receiving less potash salts. Plants treated with potash have a larger growth than plants not treated, consequently in spite of the fact that the content of chlorophyll per gram of leaf is less, each plant actually has more chlorophyll in it. Leaves of plants fertilized with potash possess the power to assimilate for a greater length of time than do those not fertilized.

In studying the effect of nitrate applications upon the hydrocyanic acid content of sorghum, PINCKNEY (**4**), found that nitrogen affected the size and the color of the plants to such an extent that sorghum may be used as an indicator of the amount of available nitrogen in the soils. Plants grown in soils containing readily available nitrogen grew rapidly and were much greener in color than plants grown in soils which did not contain available nitrogen.

Sir JOHN RUSSELL (**6**) has discussed the effects of nitrogen, phosphorus and potash upon plant growth. Nitrogenous fertilizers increase the rate of leaf growth and so produce larger leaves and stems. Also, they induce a greater formation of green coloring matter, giving darker green crops. Phosphates on the other hand greatly increase the root growth.

Methods and materials

The methods used for extracting and separating the pigments were those described in a previous paper (**11**) by the writer. The amount of chlorophyll present was measured by the colorimetric method outlined in the paper on the quantitative determination of chlorophyll (**12**). The carotinoids were estimated by means of the colorimetric methods (**9, 10**), devised by the writer. The spectrophotometric methods were not used here for they had not been developed at the time the work described in this paper had been completed.

The materials used were collected from various field fertilizer experiments of this department. The cotton leaves were from the field experiments of J. J. SKINNER and the potato leaves from those of B. E. BROWN. An attempt was always made to collect representative samples of each fertilizer plot. The samples were forwarded in waxed paper, to

prevent drying of the sample, to Washington, D. C., and the analysis was completed in this laboratory.

The numbers 1, 16, 21, 7, 8, 9, and 10 in the tables refer to that portion of the P. N. K. fertilizer triangle, in use in this department, from which the samples were taken. The composition of these fertilizers is shown in the following table I.

TABLE I

COMPOSITION OF FERTILIZERS USED IN THESE STUDIES

TREATMENT NUMBER	FORMULA		
	NH_3	P_2O_5	K_2O
1	0	20	0
16	0	0	20
21	20	0	0
7	0	8	12
8	4	8	8
9	8	8	4
10	12	8	0

The elements N, P, K, and S, were determined by the following methods, which need not be described in detail here. Total nitrogen was determined by the Kjeldahl method as modified by GUNNING and ARNOLD while total phosphorus was estimated by the NEUMANN-PEMBERTON method. Sulphur was estimated by the fusion method, and potash was estimated by the Assoc. of Offic. Agr. Chemists' method substantially as given in the year 1919.

Results

The pigment results were all obtained by a direct comparison with Lovibond slides, the readings from which were later interpreted in terms of grams of pigment. Part of the leaves were dried and part of them were extracted in the fresh condition. For the chemical analysis the leaves were all dried according to regular laboratory procedure. The dried leaves for extraction were then ground to a fine powder with sand in a ball mill before extraction.

In general, a survey of tables II and III shows that the plots fertilized with a mixture high in phosphorus produced leaves which contained less chlorophyll than did leaves from plots high in potash or nitrogen. Leaves from plots high in nitrogen contained more of the chloroplast pigments than did the leaves from plots from other parts of the triangle.

Plants fertilized with a mixture high in nitrogen always produced plants whose leaves were also high in nitrogen. The plants which were fertilized

TABLE II

ANALYSIS OF DRIED COTTON LEAVES[1] COLLECTED AT NEWBERN, NORTH CAROLINA, JULY 23, 1919

NUMBER OF PLOT	1	16	21	7	8	9	10
Chlorophyll (α+β)	21.0	26.8	29.4	28.6	22.4	31.8	31.8
N	4.97	5.22	6.03	5.14	5.09	5.31	5.50
P	0.72	0.52	0.50	0.60	0.61	0.49	0.50
K	1.34	1.88	2.09	1.37	1.86	1.99	2.33
S	1.35	1.32	1.18	1.43	1.31	1.44	1.23
Pounds of cotton per acre	1424.0	1080.0	2176.0	1434.0	1802.0	2262.0	2528.0
COLLECTED AT FLORENCE, SOUTH CAROLINA, JULY 26, 1919							
Chlorophyll (α+β)	21.0	25.0	26.0	17.6	27.6	25.0	25.0
N	2.98	3.30	5.25	3.13	3.48	4.60	4.91
P	1.03	0.52	0.32	0.37	0.34	0.48	0.51
K	0.89	1.79	1.15	1.68	1.54	1.25	1.17
S	1.52	0.96	1.05	1.49	1.53	1.17	1.05
Pounds of cotton per acre	644.0	770.0	1662.0	888.0	1426.0	1948.0	1850.0
PEE DEE EXPERIMENTAL STATION COLLECTED AT FLORENCE, SOUTH CAROLINA, AUGUST 29, 1919							
Chlorophyll (α+β)	22.4	23.2	31.8	20.2	18.4	29.4	31.0
N	2.71	3.12	4.62	2.50	2.46	2.31	3.82
P	0.61	0.48	0.36	0.50	0.37	0.31	0.32
K	1.70	2.70	1.14	1.86	1.44	0.98	1.03
S	1.18	1.09	0.74	0.95	0.64	0.62	0.84
COLLECTED SEPTEMBER 21, 1919							
Chlorophyll (α+β)	24.2	15.8	22.0	16.6	23.2	17.6	26.8
Pounds of cotton per acre	1780.0	1640.0	2280.0	1372.0	1676.0	2150.0	2130.0

[1] The results reported for the chloroplast pigments are given in milligrams per 10 grams of fresh leaves. The chemical analyses are reported in grams of N, P, K or S per 100 grams of dry leaves, *i.e.*, in per cent. Data obtained for the carotinoids are not given, for drying the leaves renders these data worthless.

with a mixture high in phosphorus always produced plants whose leaves were high in phosphorus. Plants which were fertilized with a mixture high in potash always produced cotton plants with leaves high in potash. Generally the leaves of plants high in nitrogen contained the least sulphur while those high in phosphorus contained the most sulphur.

TABLE III

ANALYSIS OF FRESH COTTON LEAVES, PEE DEE EXPERIMENT STATION. COLLECTED AT FLORENCE, SOUTH CAROLINA, JUNE 25, 1920

NUMBER OF PLOT	1	16	21	7	8	9	10
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Carotin	0.66	0.90	0.86	0.57	0.63	0.76	0.76
Xanthophyll	1.20	1.70	2.00	2.00	1.50	1.50	1.30
Total carotinoids	1.86	2.60	2.86	2.57	2.13	2.26	2.06
Chlorophyll ($\alpha+\beta$)	19.0	26.8	27.4	22.0	23.4	23.0	23.4
COLLECTED SEPTEMBER 29, 1920							
Carotin	0.70	0.78	0.90	0.78	0.86	0.86	0.75
Xanthophyll	1.60	1.03	1.16	1.00	1.30	1.50	1.16
Total carotinoids	2.30	1.81	2.06	1.78	2.16	2.36	1.91
Chlorophyll ($\alpha+\beta$)	16.6	14.8	16.6	13.6	14.8	14.8	16.00
Pounds of cotton per acre	1520.0	1040.0	938.0	1600.0	1608.0	2004.0	1620.0

In general, it may be concluded that plots high in potash gave low yields of cotton while those high in nitrogen produced the most cotton per acre, although this is not true for all types of soil. The phosphorus plots produced yields which were between those for potash and nitrogen.

A summary of the results from the potato plots (table IV) shows that the plots fertilized with a mixture high in phosphorus produced leaves which contained less chlorophyll than did leaves from plots high in nitrogen. Leaves from plots high in potash evidently contained less chloroplast pigments than did leaves from the other plots.

Potato plants fertilized with a mixture high in nitrogen produced plants whose leaves were high in nitrogen. Plants which were fertilized with a mixture high in potash produced plants whose leaves contained the least potash. Plants fertilized with a mixture high in phosphorus apparently produced leaves which were also high in phosphorus. The amount of sulphur in the leaves could not be correlated with the amount of nitrogen, phosphorus, or potash present in the leaves or that added to the soil as fertilizer.

In table V results are given for a fertilizer experiment at Arlington Farm. In this case, the results are not at all in conformity with what might have been expected and the chloroplast pigments cannot be correlated in any way with the amount of fertilizer added. However, the table shows that as the plants matured the total carotinoids increased from 2.5 to 3.27 mg. as also did the total chlorophyll from 12.8 to 15.3 mg. in each

TABLE IV

ANALYSIS OF POTATO LEAVES, DRIED BEFORE EXTRACTION

IRISH COBBLER VARIETY

COLLECTED MAY 29, 1919, NORFOLK, VIRGINIA

NUMBER OF PLOT	1	16	21	7	8	9	10
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Chlorophyll (α + β)	15.5	15.9	18.0	.....	.....	.....	.....
COLLECTED JUNE 17, 1919, NORFOLK, VIRGINIA							
Chlorophyll (α + β)	16.8	15.2	21.1	18.5	16.4	15.9	19.4
Yield (bushels per acre)	81.0	73.0	97.0	75.0	178.0	193.0	167.0
GREEN MOUNTAIN VARIETY							
COLLECTED JULY 15, 1919, RIVERHEAD, LONG ISLAND							
Chlorophyll (α + β)	26.0	19.2	32.8	21.0	28.6	35.2	31.8
Yield (bushels per acre)	176.0	191.0	91.0	166.0	192.0	181.0	187.0
NORCROSS VARIETY							
COLLECTED AUGUST 4, 1919, PRESQUE ISLE, MAINE							
Chlorophyll (α + β)	12.6	32.8	35.2	39.6	35.2	34.6	38.6
N	4.87	4.60	6.02	4.69	5.15	5.44	6.23
P	0.35	0.27	0.36	0.32	0.21	0.31	0.39
K	2.63	2.08	2.37	2.06	2.09	1.81	.....
S	0.35	0.34	0.36	0.33	0.33	0.41	0.39
No yields available							
IRISH COBBLER VARIETY							
COLLECTED AUGUST 4, 1919, PRESQUE ISLE, MAINE							
Chlorophyll (α + β)	19.2	14.0	18.4	14.0	26.0	14.8	20.2
N	4.49	5.15	6.00	4.71	5.10	5.91	5.41
P	0.37	0.30	0.26	0.29	0.29	0.39	0.34
K	2.34	2.25	2.53	3.83	3.75	3.10	1.45
S	0.55	0.72	0.63	0.45	0.71	0.40	0.42
Yield (bushels per acre)	165.0	192.0	153.0	219.0	328.0	336.0	288.0

ten gram sample of the fresh leaves. The samples of leaves which were dried show that the amount of carotin and xanthophyll lost in drying was quite large and consequently the results are unreliable, while the loss of

TABLE V

CHLOROPLAST ANALYSIS OF FRESH POTATO LEAVES WHICH WERE COLLECTED AT DIFFERENT STAGES OF GROWTH GROWN AT ARLINGTON FARM, VIRGINIA

PLOT NUMBER	DATE OF COLLECTION	1	16	21	7	8	9	10	CHECK	CHECK DRIED
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
CAROTIN	August 9, 1920	0.83	0.83	0.82	0.61	0.66	0.80	0.86	0.27	0.41
	August 24, "	0.66	0.73	0.70	0.70	0.70	0.76	0.76	0.72	.....
	September 7, "	0.70	0.63	0.15	0.66	0.93	0.74	0.68	0.88	0.56
	September 20, "	0.70	0.70	0.90	0.83	0.70	0.73	0.80	0.93	.....
XANTHOPHYLL	August 9, 1920	1.60	2.00	1.60	2.20	2.00	2.00	1.80	1.90	1.00
	August 24, "	1.60	1.70	1.60	1.70	1.60	1.10	1.50	1.20	.....
	September 7, "	2.10	2.70	3.50	1.40	1.90	1.80	2.10	2.40	1.30
	September 20, "	1.80	2.60	2.00	2.50	2.50	3.00	2.50	3.00	.....
TOTAL CAROTINOIDS	August 9, 1920	2.43	2.83	2.42	2.81	2.66	2.80	2.66	2.17	1.41
	August 24, "	2.26	2.43	2.30	2.40	2.30	1.86	2.26	1.92	.....
	September 7, "	2.80	3.33	3.65	2.06	2.83	2.54	2.78	3.28	1.86
	September 20, "	2.50	3.30	2.90	3.33	3.20	3.73	3.30	3.93	.....
CHLOROPHYLL ($\alpha+\beta$)	August 9, 1920	13.8	12.8	11.8	12.8	12.6	14.8	12.6	11.6	7.9
	August 24, "	11.3	15.6	13.1	11.8	9.5	20.1	12.6	10.9	.....
	September 7, "	14.4	14.8	12.7	11.0	15.2	12.7	14.5	16.2	13.6
	September 20, "	15.4	15.4	15.4	15.2	15.4	16.6	15.4	14.1	.....

the chlorophyll was much less. This fact shows that the leaves should never be dried if accurate quantitative data are desired for the chloroplast pigments. This table shows also that certain fertilizers do not always produce certain effects upon the development of the chloroplast pigments. The effect then apparently is due to some factor which the fertilizer influences indirectly. Here a potash fertilizer produced plants high in chlorophyll while nitrogen produced plants relatively low in chlorophyll.

The experiments with potatoes, reported in this paper, show that high yields of tubers are correlated with heavy potash fertilization, which also is correlated with a low chlorophyll content of the leaves. Low yields of tubers are correlated with high nitrogen fertilization and a high chlorophyll content of the leaves. High phosphate produces yields of potatoes which are less than those from the high potash plots and greater than those from the high nitrogen plots.

Conclusions

From the above experiments it is clear that only fresh leaves should be used in the determination of the four chloroplast pigments. A very large percentage of carotin and of xanthophyll is lost on drying. The process of drying the leaves is far more destructive to the carotinoids than it is to the chlorophylls.

Phosphorus, potash and nitrogen each may be correlated with an effect on the formation of the chloroplast pigments. Nitrogen was found to be correlated with an increase in the amount of chloroplast pigments which were present in each ten gram sample of fresh green leaves. The nitrogen was also correlated with an increase in the amount of the carotinoids. In potatoes, high potash evidently suppressed chloroplast pigment formation while this was also true in some of the plots of cotton. High phosphorus plots would then evidently produce more chloroplast pigments than potash and less than nitrogen.

Either cotton or potato plants fertilized with a mixture high in nitrogen always produces leaves with a high nitrogen content. Potato plants fertilized with a mixture high in potash produced leaves which contained the least potash, while cotton plants produced leaves which contained the most potash. Both cotton and potato plants which were fertilized with mixtures high in phosphorus also produced leaves which were high in phosphorus. In the case of potatoes the amount of sulphur in the leaves could not be correlated with the amount of nitrogen, potash or phosphorus while in the case of cotton the leaves high in nitrogen contained the least sulphur, and the leaves high in phosphorus contained the most sulphur.

Plots which were high in potash gave low yields of cotton while those high in nitrogen produced the most cotton per acre although this is not true

for all types of soil. High yields of potatoes are correlated with heavy potash fertilization which also is correlated with a low chlorophyll content of the leaves. Low yields are correlated with high nitrogen fertilization and a high chlorophyll content of the leaves.

When the results from a fertilizer experiment at Arlington Farm are correlated with results elsewhere it is seen that P, N, and K do not always have the same effect on the chloroplast pigments. At Arlington Farm the potash did not seem to suppress the chlorophyll formation nor did nitrogen increase chlorophyll formation, which is not in agreement with findings elsewhere.

The results given in this paper are in harmony with those of MAIWALD for potatoes. He found that potato leaves from plots fertilized by the addition of potash contained considerable less chlorophyll than leaves of potato plots to which no potash was added. The amount of the chlorophyll rapidly decreased in the potash plots from June to August. In the plots without potash the decrease in the chlorophyll was not so rapid. Leaves from plots fertilized with both potash and nitrogen showed a decided increase in their chlorophyll content. Plants in these plots did not show a decrease in chlorophyll from June to August as did the potash plots.

DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

LITERATURE CITED

1. CHIBNALL, ALBERT CHARLES. Diurnal variation in the total nitrogen content of foliage leaves. Ann. Bot. **37**: 511–518. 1923.
2. HENRICI, MARGUERITE. Chlorophyllgehalt und Kohlensäure-Assimilation bei Alpen- und Ebenen-pflanzen. Verh. naturf. Ges. Basel **30**: 43–136. 1919.
3. MAIWALD, K. Wirkung hoher Nährstoffgaben auf den Assimilationsapparat. Angew. Bot. **5**: 33–48, and 49–74. 1923.
4. PINCKNEY, R. M. Effect of nitrate applications upon the hydrocyanic-acid content of sorghum. Jour. Agr. Res. **27**: 717–723. 1924.
5. REMY, TH., and LIESEGANG, H. Untersuchungen über die Rückwirkungen der Kaliversorgung auf Chlorophyllgehalt, Assimilationsleistung, Wachstum und Ertrag der Kartoffeln. Landw. Jahrb. **64**: 213–240. 1926.
6. RUSSELL, SIR JOHN. Soil improvement. Fertilizers and their uses. Jour. Min. Agr. **31**: 217–223. 1924.
7. SCHAEFER, ERNST. Studien über einige Staudeneigenschaften der Kartoffelpflanze. Jour. Landw. **72**: 163–195. 1923.

8. SCHERTZ, F. M. A chemical and physiological study of mottling of leaves. Bot. Gaz. **71**: 81–130. 1921.
9. ————. The quantitative determination of carotin by means of the spectrophotometer and the colorimeter. Jour. Agr. Res. **26**: 383–400. 1923.
10. ————. The quantitative determination of xanthophyll by means of the spectrophotometer and the colorimeter. Jour. Agr. Res. **30**: 253–261. 1925.
11. ————. The extraction and separation of chlorophyll ($\alpha + \beta$) carotin and xanthophyll in fresh leaves preliminary to their quantitative determination. Plant Physiol. **3**: 211–216. 1928.
12. ————. The quantitative determination of chlorophyll. Plant Physiol. **3**: 323–334. 1928.
13. URBAN, J. Über die Farbe des Rübenkrautes früh- und spätreifender Rüben. Zeitschr. Zuckerind. Böhmen **42**: 281–297. 1918.
14. VILLE, GEORGES. Recherches sur les relations qui existent entre la couleur des plantes et la richesse des terres en agents de fertilité. Compt. Rend. Acad. Sci. Paris **109**: 397–400, 628–631. 1889.
15. WEISSMANN, H. Über den Einfluss des Kaliums auf die Entwicklung der Pflanzen und ihren morphologischen und anatomischen Bau bei besonderer Berücksichtigung der landwirtschaftlichen Kulturpflanzen. Zeitschr. Pflanzen-Düngung 2: 1–79. 1923.
16. WLODEK, JEAN. Recherches sur l'influence des engrais chemiques sur le coefficient chlorophyllien. Bull. Acad. Polon. Sci. Let. Cl. (B) Sci. Nat. **21**: 19–52. 1920.
17. ————. Recherches sur l'influence de la lumière et des engrais chemiques sur le coefficient chlorophyllien. Bull. Acad. Polon. Sci. Let. Cl. (B) Sci. Nat. **22**: 143–190. 1921.

THE EFFECT OF TEMPERATURE ON FLOWERS

F. M. ANDREWS

(WITH ONE FIGURE)

This paper is concerned with the opening and closing processes in the flowers of *Crocus* and *Tulipa*. As is well known from the work of PFEFFER, the flowers of *Crocus* and *Tulipa* are only slightly photonastic. They are, however, extremely thermonastic. As regards this thermonastic response *Crocus* is much more sensitive than *Tulipa*. In the case of *Crocus*, the temperature may be so low that the flowers cannot open, yet the flowers may develop fully, nevertheless. Thus PFEFFER found that in temperatures below 8° C. the flowers developed but did not open. He found that the flowers of some *Crocus* plants opened to an extent with fluctuations of only 0.5° C. He made this thermonastic movement more evident by means of a fine silver wire which projected beyond the flower division and which was fastened to the latter by means of lacquer. Others have found that a very fine glass capillary will serve this purpose equally well.

My experiments show that the flowers of one species of *Crocus* are capable of responding to a somewhat smaller temperature change than 0.5° C. This is the case with the flowers of *Crocus vernus* which has been found to be the most sensitive to temperature changes of any of the species of *Crocus* thus far investigated. To test this point a study of eight species of *Crocus* has been made. The "constant temperature room" has rendered excellent and trustworthy service, but it is not so easily nor so closely controlled as the device here described which has been constructed for this study. (See fig. 1.) This apparatus consisted of a bell-jar, A, of wide but low form, with an opening at the upper end for the insertion of a very sensitive thermometer, B, wires for the electric current, C, and a suitable stopper, D, for adjusting and holding these in place. A pair of bell-jars similar to the one just mentioned, the outer being somewhat larger than the other so as to cover the inner one, and held in place by a wire adjustment is even better under certain conditions. Or, an ordinary double-walled bell-jar serves the purpose very well where special precautions are necessary. In this case, however, provision must be made for the entrance of the electric wires under the base of the jar. In the low form of bell-jar shown in fig. 1, the electric wires encircle the interior at the bottom and are held in place by means of perforated perpendicular glass rods or posts, E. These glass supports are of such length as to allow several coils of number 20 nichrome heating wire, F, to be used. It was necessary to determine exactly before beginning the experiment what adjustments were required to raise and

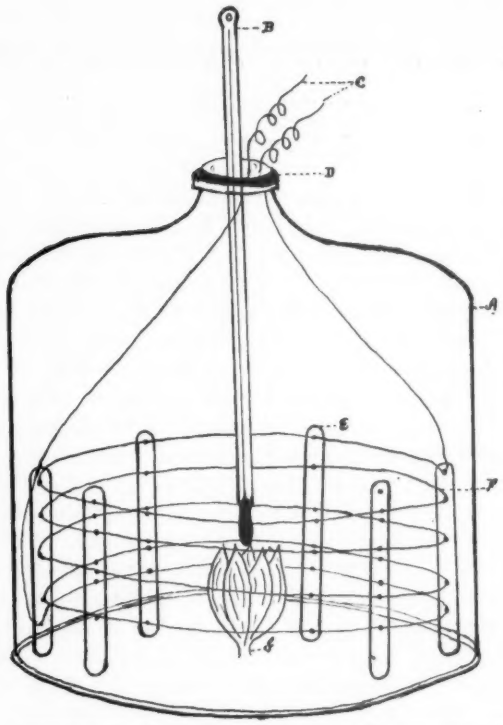


FIG. 1. Apparatus for demonstrating thermonastic responses of flowers.

maintain the temperature of the bell-jar to the desired point. With this apparatus the necessary heat for causing the opening movements of the flower segments of *Crocus* and *Tulipa* was readily produced by electricity. When the current was passed through the coils of nichrome wire, fig. 1, the necessary temperature was slowly or quickly produced according to the adjustment made. This was accomplished very exactly by using an electric current of 110 volts which was controlled by a lamp resistance in series with an adjustable wire rheostat. This arrangement also allowed the experiments to be performed either on potted plants, or better, on plants grown in the open and at any desired distance from the source of the current by increasing the length of the conducting wire. In this way the apparatus could be quickly placed over plants growing in flower beds. When it was observed that the flowers were in a probable condition for opening, the bell-jar, arranged as shown in figure 1, could often be placed over a considerable number of buds or flowers of *Crocus*. If the buds and flow-

ers of *Crocus*, G, are in an active condition, they can be caused to open or close respectively 12 times in immediate succession by alternately warming, or cooling to an appropriate degree, suitable buds or flowers on cool days. In order to cover a considerable number of buds or flowers by the bell-jar, consideration should be given previously to the planting of *Crocus* for such experimentation. This plant will bear rather close setting of its corms without detriment.

For the study of the opening and closing processes in *Tulipa* much taller bell-jars than the one shown in fig. 1 must be used, and the wire arrangement for heating increased and placed on the interior of the bell-jar in accordance with the height of the flowers. Although, as stated, *Crocus* is only feebly photonastic, still some objection has been offered to certain experimentation carried out with potted plants of *Crocus* in a room. The apparatus here described, effectively removes this objection, since it brings to bear on the plants enclosed by the bell-jar only the temperature effect, and leaves the influence of light out of doors unaffected. The other *Crocus* plants in the experimental plot therefore, serve in this connection as controls. This apparatus when used on *Crocus* and *Tulipa* in the open has also the additional advantage that the plants are under somewhat more natural conditions in other respects than is the case with potted ones. By the use of this apparatus ROYER'S idea that transpiration effects the flower movements is disproved, since this condition by my apparatus is controlled. These experiments are best carried out on cool days since then all the flowers out of doors are closed, while those that are in a responsive state under the electrically warmed bell-jar, can be caused to open. Caution should at all times be observed, to select flowers which have attained the proper stage of development. This is sometimes difficult to determine, or can be ascertained only by their failure to respond to the temperature changes. The flowers may be too immature, but more often they are too old and hence have passed, with respect to temperature, their perceptive condition. After a *Crocus* or *Tulipa* bud has been opened by warming under the bell-jar, then by removing the bell-jar or otherwise filling it with cool air, the open flowers will close. This occurs more quickly in *Crocus* than in *Tulipa*. The opening and closing of the flowers in both *Crocus* and *Tulipa* also take place rapidly or slowly according to the changes in temperature, within their responsive ranges. A slight fullness of the *Crocus* bud indicates a condition for opening, although this may be deceptive. Single flowers may be made to open very rapidly, if placed, as PFEFFER points out, in warm water after removal from a cool place. These flowers, however, rarely stand upright in the water, which makes some observations of their behavior less obvious. This difficulty may be overcome by enclosing the lower end of the perianth tube in a rather closely fitting glass tube of proper weight and

length, so that part of the glass tube's weight and lower end rests lightly on the bottom of a wide crystallizing dish in which the warm water is only a few cm. in depth. By this method a good many flowers may be observed simultaneously and the extreme opening process may be followed to good advantage.

By means of the electrically warmed bell-jar described here, flowers of *Crocus vernus* experimented upon out of doors were caused in many instances to open to an extent by increasing the temperature by only 0.2° C. The flowers of *Tulipa*, as is well known, are less responsive and no flowers of this plant were found that showed any tendency to open with a change of less than 1° C. In both of these plants the response that did occur in the flowers was much slower than when the temperature was higher or more favorable in the warmed air of the bell-jar or in the warm water. These experiments show that the flowers of both *Tulipa* and *Crocus* are much more sensitive to temperature changes than has been observed heretofore for these plants.

DEPARTMENT OF BOTANY,
INDIANA UNIVERSITY.

WILHELM PFEFFER
1845–1920

BRIEF PAPERS

WILHELM PFEFFER

(WITH ONE PLATE AND ONE FIGURE)

WILHELM PFEFFER was the son of an apothecary. He was born the 9th of March, 1845, in Grebenstein, near Cassel, Germany. He received his early education in Grebenstein and later attended the schools of Cassel.[1] He evinced in early life a wide and keen interest in various departments of science in which his information, even when yet a boy, was very unusual. At first, he was much interested in collections of various kinds. This work took him on many trips, especially in the Alps mountains. His investigations of the plants of this region led to the publication of his well known Bryographical Studies dating from 1867 to 1869.

As a university student PFEFFER's training was extensive. He studied at the university of Göttingen and received the degree of Doctor of Philosophy there in 1865. Afterward, he studied at the universities of Marburg, Würzburg, and Berlin. At Würzburg PFEFFER was a student of JULIUS VON SACHS. There he was associated with many other investigators who afterwards became distinguished workers in their fields: KLEBS, DE VRIES, GOEBEL, BREFELD, NOLL, STAHL, KRAUS, MÜLLER-THURGAU and many others. PFEFFER's preparation in physics and chemistry was very extensive, and the mastery of these sciences was of great service to him in his work in plant physiology.

As a teacher, PFEFFER's experience was also wide. He began his career formally as an academic teacher in the university of Marburg in 1871. He was professor in the university of Bonn in 1873; in the university of Basel in 1878; in the university of Tübingen from 1878 to 1887 and finally professor in the university of Leipzig from 1887 till his death, which occurred January 31, 1920.

His lectures were largely attended. On these he spent much time and frequently produced interesting and valuable demonstrations of plant activities by means of ingeniously contrived apparatus. Many of these experiments were extremely difficult to show to a large audience due to the special conditions which must not infrequently be provided.

In the laboratory, PFEFFER was careful and exacting. On the other hand his criticisms were always made in a most friendly and helpful manner. No work or trouble on his part was too great to help or encourage

[1] Some details in this paper have been taken or verified from Professor FITTING'S account of PFEFFER. Ber. deutsch. bot. Ges. **38**: 30–63. 1920.

any student who was industrious and determined to work. His great ability to understand and attack a problem in the best and most direct manner was astonishing, even to those of extensive training and experience. A very accurate memory nearly always enabled him to make an instant and exact reference to the needed literature. His keen insight and ability together with his long and successful experience gave him a grasp of the subject that was at once noted as unusual by all who came in contact with him.

PFEFFER's contributions to science number nearly one hundred. Among these are some papers which occupy comparatively few pages. However, what short papers he did write are very compact and to the point; for these, like all of his papers, teemed with new facts, to each of which he gave careful study. They frequently represented long periods of experimentation. In these papers, as elsewhere, he shows his ability to go directly to correct conclusions, and where necessary to summarize with great clearness and brevity. Other papers were much longer due to the nature of the topic under discussion. Many of his contributions were so extensive that they were published in book form. The second edition of his Pflanzenphysiologie is the largest contribution of this nature. PFEFFER's studies on osmosis (1877) are classical, and have had a wide bearing in many fields of science. The physico-chemical importance of PFEFFER's osmotic studies have been amply indicated by VAN'T HOFF in his well known studies of osmotic pressure, gas pressure and solutions. Mention should also be made of PFEFFER's Physiologische Untersuchungen (1873) which constitute one of his best known investigations. His studies of leaf movements are very extensive and cover a long period of investigation. The skillful methods used in these studies and the valuable results obtained constitute a very important advance in this field. His work on chemotaxis (1888) as well as his Studien über Energetik (1892) are of great interest and importance. These and many others of PFEFFER's valuable contributions to plant physiology could be mentioned if space allowed their enumeration. His ability to present and summarize the vital points of a subject is well shown in his Pflanzenphysiologie (1897–1904). In this work, a huge field of literature had to be carefully sifted and summarized and the results brought together in a connected account. The writing of this work was therefore an enormous task and it constitutes the greatest production of its kind in the field of plant physiology. It gives ample proof of PFEFFER's thorough grasp of the subject and shows his exceptional qualifications and his ability to successfully execute this difficult undertaking. Even if PFEFFER had written only his Pflanzenphysiologie, science would be deeply indebted to him, and PFEFFER, aside from any of his other distinguished work, would be entitled to a place of distinction.

In 1915 a "Festschrift" for PFEFFER was issued in honor of his seventieth birthday and the fiftieth year since he received his Ph.D. degree. This book also constitutes the 56th volume of the Jahrbücher für wissenschaftlichen Botanik of which PFEFFER was editor. After the death of his teacher, N. PRINGSHEIM, in 1895, PFEFFER, in conjunction with E. STRASBURGER, became editor of this well known journal. After the death of STRASBURGER in 1912 PFEFFER carried the burden of this task alone. This "Festschrift" is composed of papers written expressly for this volume by his former students in various countries. This volume also contains a list of PFEFFER's papers, beginning with his thesis which appeared in 1865, to and including the year 1915, although at that time his last book on sleep movements which appeared in 1915 is not listed. In this volume is also included a list of 260 of his former students. As a distinguished plant physiologist and as an organizer of the subject PFEFFER is unequaled. The nearest approach to him in these respects was JULIUS VON SACHS. This volume contains his photograph from which plate IV has been reproduced.

Aside from his regular work, he had some time to spend with his students. They were invited once each semester to his home. Moreover, he always presided at the botany club which held its meetings every two weeks, in the evening. In addition to the paper of the meeting the special privilege was granted to each person, before the meeting, to place in a box a botanical question for PFEFFER to answer. These questions he always answered, even without previous notice or preparation, without hesitation and very clearly and completely.

Fig. 1 is a photograph of the Botanical Institute where PFEFFER was director and professor. It was designed and build by A. SCHENK, the pre-

FIG. 1. Botanisches Institut, Leipzig.

decessor of PFEFFER as professor of botany at Leipzig. This photograph is taken from a paper written by Professor PFEFFER especially for the celebration of the 500th anniversary of the University of Leipzig.[2] It faces east which is the view shown here. It will be a familiar sight to those who have studied there. It will also give those who have not studied there an idea of the place from which so many of PFEFFER's important contributions to plant physiology have come. The building stands in the botanical garden. The basement of the building is chiefly used as special experimental rooms. The first floor is devoted exclusively to laboratories with the exception of the library and lecture room. The top floor serves as a residence for the Director. PFEFFER could take 20 research students at a time in this Institut. The fame of the Leipzig Institut was so unusual, with PFEFFER as its director, that it was necessary to make arrangements a long time in advance if one expected to secure a place. In addition there was generally a considerable waiting list. All of PFEFFER's many students, now scattered everywhere in so many different countries, count it a special privilege that they were fortunate enough to study under his direction. On account of PFEFFER's wide and successful career both as an investigator and a teacher he has done more than any one else to train and inspire the plant physiologist of the present day.—FRANK M. ANDREWS, *Indiana University.*

A SIMPLE METHOD FOR NITRATE NITROGEN DETERMINATION IN WHEAT PLANTS[1]

The greatest difficulty in getting satisfactory results in nitrate nitrogen determinations in plant material is due to the presence of the large amounts of soluble and colloidal organic substances, or to the difficulty in removing these substances before the determinations are made. When nitrates are determined by reduction and subsequent distillations, fairly large samples are necessary and the interference of the decomposition of the organic materials during the distillation makes the determination somewhat unsatisfactory. Likewise the colorimetric method is impossible unless these soluble organic substances are removed before evaporation. The removal of these substances would be equally desirable in the distillation method.

[2] This paper, entitled Die botanischen Institute, as well as the photograph used here, were presented to the writer by Professor PFEFFER.

[1] Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper no. 152, College of Agriculture and Experiment Station, State College of Washington, Pullman, Washington.

TABLE I

COMPARISON OF THE GILBERT AND SIMPLE METHOD OF DETERMINING NITRATE NITROGEN IN TWO VARIETIES OF WHEAT

VARIETY	GILBERT METHOD				SIMPLE METHOD		
	SAMPLE	NO_3 N	AVERAGE	RECOVERED	NO_3 N	AVERAGE	RECOVERED
	gm.	*mg.*	*mg.*	*per cent.*	*mg.*	*mg.*	*per cent.*
Jenkins Club	0.2	0.3600	0.3600		0.3600	0.3600	
		0.3600			0.3600		
Jenkins Club, plus 0.1 mg. NO_3 N	0.2	0.4000	0.4000	40	0.4600	0.4600	100
		0.4000			0.4600		
Hybrid 128	0.2	0.0500	0.0525		0.0500	0.0500	
		0.0550			0.0500		
Hybrid 128, plus 0.1 mg. NO_3 N	0.2	0.1000	0.0975	45	0.1450	0.1400	90
		0.0950			0.1350		

In an experiment where a large number of nitrate nitrogen determinations were to be made on eleven varieties of wheat in different stages of development, an attempt was made to select the simplest and most efficient method for removing the interfering organic substances preliminary to the phenol disulfonic acid treatment.

The method for clearing the solution, reported by BURRELL and PHILLIPS (**1**), in which lead acetate was used to precipitate the soluble matter, was tried but considered too long and somewhat difficult. The method used by GILBERT (**2**) was next tried in which carbon black is substituted for lead acetate, and was found much simpler for this purpose. The cleared solution in both methods contained a large amount of soluble salts which prolonged the evaporation of the solution. This was found to be caused by the addition of the magnesium carbonate. In order to eliminate the magnesium carbonate, the following method, which has proven to be very satisfactory, was evolved.

Two tenths gram of plant material which had been dried according to the method reported by THOMAS (**3**) was weighed out, transferred to a 250-cc. beaker and leached with 74 cc. of distilled water for an hour. After leaching, 5 cc. of saturated silver sulphate ($AgSO_4$) was added, followed by 1 cc. of normal copper sulphate ($CuSO_4$), 0.2 gm. of calcium hydroxide ($Ca(OH)_2$ powdery), and 2 to 3 gm. of carbon black. This extract containing the plant material was stirred after each addition of the above solutions and salts, and filtered. One half of the filtrate was evaporated to dryness, and nitrates determined by the phenol disulfonic acid method. The following table gives the nitrate nitrogen content of two varieties of wheat and the recovery of nitrates added to each variety as determined by the method used by GILBERT and the one here described.

Of the several varieties analyzed for comparison, only two are presented in the table. These were selected for their difference in nitrate nitrogen content and soluble organic matter content. The data show that both methods gave duplicate results on the plant material, but for some undetermined reason the recovery of added nitrates by the GILBERT method was incomplete, while in the modified method it was all recovered in Jenkins Club and nearly so in Hybrid 128.

The advantages of this simple method over that used by GILBERT are: (1) it reduces the filtrations to one; (2) it eliminates the washing of any residue; (3) it speeds up the evaporation of the filtrate; (4) it eliminates boiling; and (5) it gives good recovery to added nitrates. The above advantages make for speed and accuracy, both of which are important factors when a large number of determinations are to be made.—H. F. HOLTZ and CARL LARSON, *State College of Washington.*

LITERATURE CITED

1. BURRELL, R. C., and PHILLIPS, T. G. The determination of nitrate nitrogen in plants. Jour. Biol. Chem. **65**: 229–234. 1925.
2. GILBERT, B. E. The adaptation of certain colorimetric methods to the estimation of nitrates, phosphorus and potassium in plant solutions. Plant Physiol. **1**: 191–199. 1926.
3. THOMAS, W. Nitrogenous metabolism of *Pyrus malus*. Plant Physiol. **2**: 55–70. 1927.

NOTES

Fifth International Botanical Congress.—At the time of the Intertional Botanical Congress held at Ithaca in 1926, it was decided to hold the next congress in England. The announcement has been made that the fifth congress will be held at Cambridge, August 16–23, 1930. The meeting will be organized in seven sections, paleobotany, morphology and anatomy, taxonomy and nomenclature, plant geography and ecology, genetics and cytology, plant physiology, and mycology and plant pathology.

The chairman in charge of the program for plant physiology is Dr. F. F. BLACKMAN, of the Botany School at Cambridge. The papers to be presented will be arranged by invitation, and the discussion will also probably be arranged by the sectional subcommittee. Membership in the Congress is to cost one pound, which should be sent to the treasurer of the Congress, Dr. A. B. RENDLE, British Museum of Natural History, London, S. W. 7., not later than April 1, 1930. The treasurer desires early notification of the intention to attend the Congress, even if membership is not paid at once. The Congress will provide a rare opportunity to meet the plant physiologists of other lands.

Adoption of the Constitution.—The ballot on the adoption of the constitution resulted in a unanimous vote favorable to adoption, and the provisions become effective immediately. This instrument will enable the American Society of Plant Physiologists to handle its affairs advantageously, and lays a foundation for financial strength and stability. Plans are in preparation to place a printed copy of the constitution in the hands of all members of the society.

Representative on the A. A. A. S. Council.—Dr. P. D. STRAUSBAUGH, Professor of Botany at the West Virginia University, has been appointed by the President to represent the American Society of Plant Physiologists on the Council of the American Association for the Advancement of Science. He fills the vacancy in the representation caused by the death of Dr. HARRIS M. BENEDICT, of the University of Cincinnati, last October.

Increased Price of Volumes I and II of Plant Physiology.—The very large demand for the early volumes of PLANT PHYSIOLOGY has almost exhausted the editions printed during 1926 and 1927. The executive committee has considered the matter carefully, and has decided to increase the price for the remaining volumes for these two years to $10 per volume. It is unfortunate that this step must be taken. The editorial board has tried to pursue a safe financial policy, printing editions no larger than could

be paid for rather promptly by the Society, but with what was considered an appropriate surplus of each issue. The unforeseen demand for the journal has depleted the surplus much more rapidly than was anticipated. While the few remaining volumes last, they can be had by addressing Dr. H. R. KRAYBILL, Department of Agricultural Chemistry, Purdue University, Lafayette, Indiana.

Portrait of Sachs.—In accordance with the announcement made in PLANT PHYSIOLOGY in October, 1928, a small number of portraits of JULIUS VON SACHS will be available for those who desire them for framing. There is something inspiring about the faces of great leaders, and laboratories decorated with the portraits of such men make a lasting impression upon students who are following in the footsteps of these forefathers of our science. The portraits of SACHS may be had in the same manner as the portraits of TIMIRIAZEFF, as announced on page 522 of Vol. 3 of PLANT PHYSIOLOGY.

Fourth Pacific Science Congress.—The fourth Pacific Science Congress, sponsored by the Pacific Science Association, is being held under the auspices of the Netherlands Indies Pacific Research Committee in Batavia and Bandoeng, Java, May 16–25, 1929. If it is possible to secure a report of this meeting, such matters as may interest plant physiologists will be presented to the readers of PLANT PHYSIOLOGY in some future number.

Seventh Colloid Symposium.—The seventh Colloid Symposium is to be held this year at Johns Hopkins University, Baltimore, Maryland, June 20–22, 1929. The guest of honor will be Dr. F. G. DONNAN, famous for his studies of colloidal equilibrium relations. Visitors expecting to attend this meeting should write to Dr. W. A. PATRICK, Department of Chemistry, Johns Hopkins University, for reservation of rooms in the University Dormitory. The number that can be accommodated in this way is limited to about 150, and cost of room and board for the meeting is but $6.00. Visiting plant physiologists will find a cordial welcome at the Laboratory of Plant Physiology, also.

International Critical Tables.—The fifth volume of this important work has been published by the McGraw-Hill Co., N. Y. This volume contains data on viscosity of gases, metals and alloys, aqueous solutions of strong, weak, and non-electrolytes, and fluidity of non-aqueous solutions; kinetics of vaporization, solution, crystallization, diffusion and permeability; specific heat of gases and vapors, liquids, and solids; thermal effects, such as latent heat of fusion, vaporization, transformation, heat of adsorption

and wetting, heat of expansion, heat of mixing of liquids, heat of solution, precipitation, and dilution, heat of neutralization of acids and bases, heat of combustion of organic compounds, and heat of formation; thermal conductivity of gases, liquids, solids, and heat transfer by convection; radiometry (heat and light) including black body radiation, thermal radiation from selected sources, efficiency of selected sources of light, reflectivity of metallic and non-metallic substances, transmissivity, spectral absorption of light and heat by inorganic and non-metallic materials, and spectral filters'; spectroscopy, including emission spectra, persistent lines, absorption spectra (visible, ultraviolet and infra-red spectra), solar spectrum, celestial spectra, low temperature luminescence, fluorescence of gases, spectral series of elements, band spectra, etc.; photometry; mechanical equivalent of light; properties of photographic materials; and properties of soaps and their aqueous solutions.

This volume is without question one of the most valuable for physiologists in the entire set. It forms an invaluable source of information for biophysical research.

The National Research Council has announced that it is impossible to present the entire work in the five volumes originally planned. This is due to the extraordinary expansion of scientific investigations during and since the late world war. Faced with the alternatives of eliminating data from unpublished chapters, or eliminating whole chapters, or expanding the work, the decision was reached to expand the work to seven volumes. Through the generosity of the Research Corporation, the Chemical Foundation, and the United States Steel Corporation, enough of the cost of the additional volumes was met to permit offering them to all subscribers at the same price per volume as the original pre-publication subscription price, $7.00 per volume. This makes the whole work to subscribers $49.00 instead of $35.00 as announced previous to the publication of the first volume. While it is unfortunate to have to change the price of the complete set to those who subscribed for it at the lesser price, it is surely wiser to increase the size and cost of the work than to eliminate any of the assembled data.

Volume six will contain the data for X-rays, electronics, electricity, electrical conductivity, electrolytic E.M.F., and magnetism. The final volume will present data on sound, refractivity, optical activity, chemical kinetics, thermodynamics, explosives, and will contain a complete index.

Physiology and Biochemistry of Bacteria.—This work by Prof. R. E. BUCHANAN and Dr. ELLIS I. FULMER of the Iowa State College is being published by the Williams and Wilkins Co., Baltimore, Md. Vol. I contains five chapters as follows: Introduction and scope of physiological bacteriol-

ogy; growth phases and growth rates in cultures of microorganisms; chemical composition of the cells of microorganisms; physico-chemical and physical characteristics of microorganisms and their environment; and energy relationships, growth and movement of microorganisms. Energetics.

The chapter on growth rates presents the facts and theories, with attention to the autocatalyst theory of ROBERTSON, but takes the sensible view that plotted curves and equations describing them are not to be used without caution in attempts to interpret the causal relationships of growth. In the higher plants we certainly have better theories than the autocatalyst theory to explain sigmoid growth curves.

The chemical composition chapter gives data on water content, elementary constitution, organic constituents, carbohydrates, fats and other lipoids, pigments, and inorganic globules, crystals and inclusions.

The final chapter considers types of energy, sources of energy for microorganisms, utilization of energy in synthesis, heat production, light production, and movement.

The work fills a real need, bringing together much valuable and scattered data. It calls attention to the gaps in our knowledge, and should stimulate investigators to more vigorous attack upon the unsolved problems of bacterial life. It repays careful reading. The price of the volume is $7.50.

Enzyme Actions and Properties.—The translation by R. P. WALTON of the treatise on Enzyme Actions and Properties by ERNST WALDSCHMIDT-LEITZ makes available in English the first comprehensive and authoritative account of the studies of the WILLSTÄTTER school on the purification and measurement of enzymes, studies in which the author played a prominent rôle. The book is divided into two parts. The first part deals with the general mathematical and physico-chemical factors,—the colloidal and electrolyte nature, specificity, reaction kinetics, activation, and methods of preparation, purification and determination of enzymes. Here WILLSTÄTTER'S adsorption methods are fully discussed.

In the second part a few typical and much-studied enzymes are taken as types of the various groups, and are considered in great detail. Castor bean lipase, malt amylase, and yeast sucrase are the plant enzymes which are treated comprehensively. In general, animal enzymes get much more attention, which only emphasizes how much more is known about them than about plant enzymes. However, quite a little English and American work seems to have been overlooked, although these omissions affect only the specific and not the general value of the work. The translator has done a good job, and usually has made smooth English. However, there is no

justification for the use of such spellings as "peroxydase" and "oxydase," or the consistent failure to capitalize generic plant names. The format is good, and the author and subject indices comprehensive. The book is 255 pages, is published by John Wiley and Sons, and retails for $4.00.

Hydrostatic-Pneumatic System of Trees.—An excellent monograph on sap ascent has been published by the Carnegie Institution as Publication no. 397, under the title, "The hydrostatic-pneumatic system of certain trees: Movements of liquids and gases." The authors are Dr. D. T. MacDougal, J. B. Overton, and Gilbert M. Smith. They discuss the liquid and gas system of species of *Salix, Quercus, Alnus, Juglans, Pinus,* and others, and give a fine presentation of the organization of woody stems. This section on organization should be the possession of every student of plant physiology. Then follow sections on the path of the transpiration stream, factors affecting localization of the stream, variations in the path, and lateral movement of water in the stems.

The later sections deal with the gas system of the woody stem, suctions and pressures and the factors affecting them, and the rôle of living cells in sap flow. The conclusions are in agreement with Dixon's cohesion theory of sap rise, and give no comfort to those who are following Bose's interpretation of sap rise. The price of this publication is $1.25 (cloth $2.25), and orders should be sent to the Carnegie Institution, Washington, D. C.

Colloid Chemistry.—A second edition of The Svedberg's Colloid Chemistry has been published by the Chemical Catalog Co., New York. This edition gives more space to the X-ray method of analysis of colloidal sols and gels than the first edition. Improvements are recorded in the technique of ultramicroscopy, and measurements of cataphoresis and diffusion. Also, the chapter dealing with the Donnan potentials (Part III) has been revised in accordance with the recognized importance of the Donnan phenomena. The book is quoted at $5.50. Orders should be sent to the publishers.

Growth.—A little book from the Yale University Press by Dr. William J. Robbins, Samuel Brody, Albert Garland Hogan, Clarence Martin Jackson, and Charles Wilson Greene considers the nature of growth, its statistical, nutritional and morphological aspects, and physiological regulation of growth. The book is a series of popular lectures, mainly on animal growth, presented before the Missouri chapter of the Sigma Xi. The price is $3.00.